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NEW AGAVES FROM SOUTHWESTERN UNITED STATES[1]

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Two specimens of *Agave* were submitted recently to the Missouri Botanical Garden by Mr. W. I. Beecroft of Escondida, California, for identification. These plants had been collected at an altitude of 4000–5000 ft. in the mountains of southwestern Nevada. They seem to accord with material in the herbarium which Dr. Engelmann had referred doubtfully to *Agave utahensis*, as a possible variety. A more careful examination shows that they differ from that species, of which the type material is in the Garden Herbarium, in several important details.

In the comprehensive studies of the Agaves of the United States by Miss A. Isabel Mulford under the careful direction of Dr. Wm. Trelease, the material studied by Dr. Engelmann was merged, possibly because of its fragmentary nature, with *Agave utahensis*. A critical comparison of these fragments and the specimens from southwestern Nevada with the type of *A. utahensis* shows the following striking differences:

[1] Issued December 30, 1929.

A. utahensis	*A. utahensis* var. *nevadensis*
Leaves strongly inrolled in the terminal portion	Leaves not strongly inrolled in the terminal portion
Terminal spine stoutish, deeply grooved, 2.5 cm. or less long	Terminal spine slender, flat or shallowly grooved on the upper surface, 5 cm. or more long
Perianth-tube very slender, 4–5 mm. long	Perianth-tube stoutish, 1–2 mm. long
Perianth-segments 10–12 mm. long	Perianth-segments 8–10 mm. long

These differences warrant the recognition of this plant at least as a distinct variety which is characterized as follows:

Agave utahensis Engelm. in Watson, Bot. King. Exp. 497. 1871.

Var. **nevadensis** Engelm.[1] in herb. Plate 32.

Stem short, thick, 3–4.5 cm. in diameter; leaves lance-attenuate, 1–2.5 dm. long, 2 cm. or less broad, dilated at base (3–5 cm.), glaucous and minutely scurfy on both surfaces; terminal spine slender, linear-attenuate, grayish, pungent, 3–6 cm. long; marginal prickles 2–4 cm. apart, flattened, grayish white, friable, mostly recurved, surrounded at the base, as in the case of the terminal spine, by a narrow dark brownish area; leaf-margins herbaceous, sinuate, becoming minutely serrulate on the upper portion of the dilated base; flowers about 2.5 cm. long, ovary 10 mm. or less long, perianth-tube stoutish, 1–2 mm. long, perianth-segments 8–10 mm. long.—California: Ivanpah, Mohave Desert, May, 1882, *S. B. & W. F. Parish 414* (Mo. Bot. Gard. Herb.), TYPE. Specimens from southwestern Nevada sent recently to the Missouri Botanical Garden by Mr. W. I. Beecroft for identification appear to belong to this variety.

Specimens in the Missouri Botanical Garden Herbarium from St. George, Utah, collected by Dr. Edward Palmer in 1877, were

[1] **Agave utahensis** Engelm. var. **nevadensis** Engelm. in herb. Perennis; caulibus brevibus, crassibus, 3–4.5 cm. diametro; foliis lanceo-attenuatis, 1–2.5 dm. longis, 2 cm. vel minus latis ad basin (3–5 cm.) dilatatis, utrinque glaucis et minutissime porriginosis; spina terminali tenui, lineari-attenuata, cinerea, 3–6 cm. longa; aculeis complanatis, cinero-albidis, friabilis, plerumque recurvatis, circa basin ferrugineis, 2–4 cm. remotis; marginibus foliorum ad basin dilatatis, integris vel serrulatis; floribus ca. 2.5 cm. longibus; ovario 10 mm. vel minus longo; tubo perianthii 1–2 mm. longo; lobis perianthii 8–10 mm. longis.—California: Ivanpah, Mohave Desert, May, 1882, *S. B. & W. F. Parish 414* (Mo. Bot. Gard. Herb.), TYPE.

mentioned by Miss A. Isabel Mulford[1] as a thick-leaved form of *Agave utahensis* Engelm. and were designated by Dr. William Trelease as "*A. utahensis* var. *nevadensis*." However, the plant which Dr. Engelmann characterized and recorded in his unpublished notes as *Agave utahensis* var. *nevadensis* was collected by S. B. and W. F. Parish at Ivanpah, Mohave Desert, in May, 1882. A further study of the Palmer plant in the light of additional material shows that it is not only specifically distinct from *A. utahensis* and its variety *nevadensis*, but apparently represents an undescribed species belonging to the section *Geminiflorae*. The following description records the outstanding characters of the species:

Agave scaphoidea[2] Greenman & Roush, n. sp. Leaves linear-lanceolate, thick and rigid, openly concave, 12–35 cm. long, 1.5–3.5 cm. broad, slightly narrowed toward the base, minutely roughened on the surface, gradually attenuate above the middle; terminal spine stout, reddish-brown, pungent, 4 cm. long, openly grooved for about one-half its length and decurrent along the leaf-margin for nearly or quite 4 cm.; marginal prickles short-triangular, slightly curved, reddish-brown, papillate, about 3 cm. apart on the otherwise straight and herbaceous leaf-margins; inflorescence spicate; flowers in pairs, 3–3.5 cm. long; ovary 10–12 mm. long; tube of the perianth 6-8 mm. long, slender; perianth-segments oblong, 15–17 mm. long, obtusish; mature fruit not seen.—Utah: St. George, May, 1877, *Ed. Palmer* (Mo. Bot. Gard. Herb. Nos. 124604 TYPE, 124605, 124606, 124607 in part).

This species has been confused hitherto with *A. utahensis* of

[1] Mulford, A. Isabel. Agaves of the United States. Rept. Mo. Bot. Gard. 7: 78. 1896.

[2] **Agave scaphoidea** Greenman & Roush, n. sp. Folia lineari-lanceolata, crassa et rigida, aperte concava, 12–35 cm. longa, 1.5–3.5 cm. lata, versus basin paulo angustata, utrinque minutissime aspera, supra mediam gradatim attenuata; spina terminali rigida, ferruginea, 4 cm. longa, plus minusve canaliculata, ad marginem folii decurrenti; aculeis brevi-triangularis, paululo curvatis, ferrugineis, papillosis, circiter 3 cm. remotis; inflorescentia spicata; floribus binis, 3–3.5 cm. longis; ovario 10–12 mm. longo; tubo perianthii 6–8 mm. longo, tenui; lobis perianthii oblongis, 15–17 mm. longis, obtusis; capsula ignota.—Utah: St. George, coll. of May, 1877, *Ed. Palmer* (Mo. Bot. Gard. Herb. Nos. 124604 TYPE, 124605, 124606, 124607 in part).

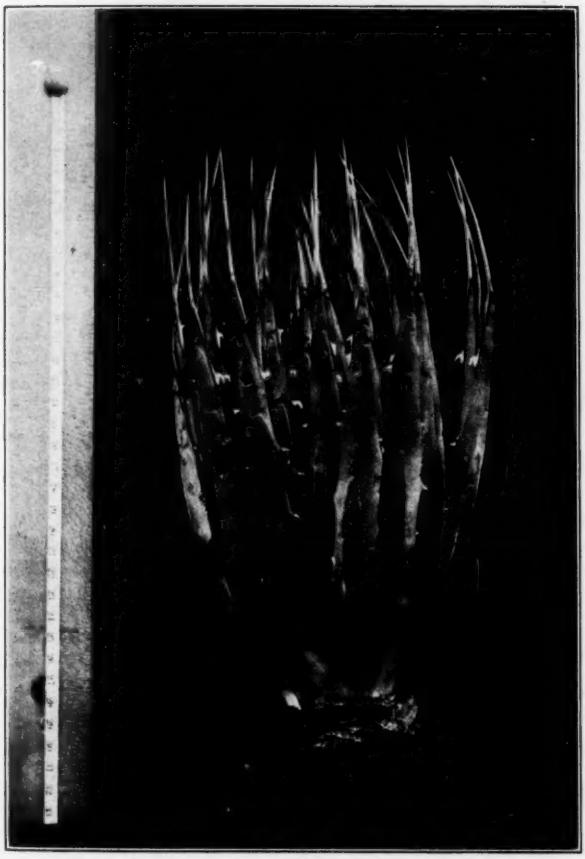
Engelm. from which, however, it is clearly distinct as shown by the following tabulated characters:

Agave utahensis	*Agave scaphoidea*
Leaf-margins sinuate	Leaf-margins straight
Prickles approximate, gray, friable	Prickles remote, reddish, papillate, rigid
Terminal spine slender, 2 cm. long, grayish, involute for practically its entire length	Terminal spine stout, 4 cm. long, reddish-brown, openly grooved for about one-half its length
Perianth-segments 10–12 mm. long, tube of the perianth 4–5 mm. long	Perianth-segments 15–17 mm. long, tube of the perianth 6–8 mm. long

Explanation of Plate

PLATE 32

Agave utahensis Engelm. var. *nevadensis* Engelm. in herb.
Nevada.
From a living plant collected by Mr. G. E. Barrett
in southwestern Nevada.



GREENMAN AND ROUSH—NEW AGAVES

STUDIES IN THE UMBELLIFERAE. II

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1. NEOPARRYA, A NEW GENUS OF THE UMBELLIFERAE

Neoparrya[1] n. gen. of the Umbelliferae. Herbaceous, acaulescent perennial. Leaves pinnatisect. Inflorescence spreading; peduncles exceeding the leaves; involucre absent; involucel of small, inconspicuous bracts. Calyx teeth persistent. Stylopodium lacking. Fruit oblong, glabrous; ribs slightly developed; oil tubes small, numerous, scattered in the pericarp; strengthening cells absent.

The type species is **Neoparrya lithophila,**[2] n. sp. Plate 33.

Seseli Nuttallii Gray, Proc. Am. Acad. 8: 287. 1870, as to Parry collection, No. 83.

Plant acaulescent, about 1.5 dm. high; leaves petiolate, oblong-lanceolate in outline, 8–10 cm. long, about 2.5 cm. broad, somewhat rigid, glabrous, ultimate segments linear, 5–20 mm. long; umbels several-rayed, rays 0.5–1.5 cm. long; involucel bracts linear-lanceolate, about 3 mm. long; styles 2–3 mm. long, persistent; fruit 3–5 mm. long, lateral and dorsal ribs inconspicuous.

Type specimen: *Dr. C. C. Parry 83*, "on rocks, Huefano Mountains, New Mexico," Sept. 1867 (TYPE in the Gray Herbarium of Harvard University; isotypes in the Missouri Botanical Garden Herbarium).

[1] **Neoparrya** Mathias, n. gen.—Herba perennis, acaula. Folia pinnatisecta. Radii umbellae expandi; pedunculi foliis longiores; involucra nulla; involucella parvae obscurae bracteae. Calycis dentes persistentes. Stylopodium nullum. Fructus oblongus, glaber; jugae obscurae; vittae parvae, multae, in pericarpio hinc inde distributae; cellae firmantes nullae.

[2] **Neoparrya lithophila** Mathias, n. sp.—Planta acaula, circa 1.5 dm. alta; foliis petiolatis, in circumscripto oblongo-lanceolatis, 8–10 cm. longis, circa 2.5 cm. latis, subrigidis, glabris, ultimis segmentis linearibus, 5–20 mm. longis; umbellis multiradiatis, radiis 0.5–1.5 cm. longis; involucellae bracteis lineari-lanceolatis, circa 3 mm. longis; stylo 2–3 mm. longo, persistente; fructibus 3–5 mm. longis, lateralibus dorsalibusque jugis obscuris.—Collected "on rocks, Huefano Mountains, New Mexico," Sept. 1867, *Dr. C. C. Parry 83* (Gray Herb., TYPE; Mo. Bot. Gard. Herb., isotypes).

Issued December 30, 1929.

This species is the *Seseli Nuttallii* Gray, Proc. Am. Acad. 8: 287. 1870, in part. Gray says in describing the species that it "has been for several years known to me in a specimen collected by Nuttall, in flower only, and presented by the kind Mr. Durand. It is ticketed by Nuttall '*Cynomarathrum saxatile*,' but it is not published. The same plant, in fruit only, was gathered by Dr. Parry in 1867, in the mountains of the northeastern part of New Mexico." The Nuttall specimen above mentioned, which is now in the Gray Herbarium, was examined in connection with this study as was also the Parry collection; the two plants are not congeneric. Watson, in the Proceedings of the American Academy 22: 475. 1887, made the same conclusion: "The plant of Parry's collection which was included with Nuttall's under *Seseli Nuttallii* remains uncertain. It is scarcely a congener of the *Seseli Hallii*, Gray, described with it. In the very ripe fruit of Parry's specimen the albumen is apparently surrounded by a thin continuous layer of resinous matter, while what appear to be empty vittae are scattered through the somewhat corky pericarp." After a critical examination of specimens of the Parry collection of 1867, No. 83, from the Gray Herbarium and the Missouri Botanical Garden Herbarium, they were found to merit generic recognition.

The genus is known only from the type locality in northern New Mexico. It was collected by Dr. Parry, in whose honor it is named, at Huerfano Peak near Servilleta, Taos County. Dr. Parry was the botanist on the Pacific Railway Expedition made from Salina, Kansas, to the Pacific. The party crossed the Sangre de Cristo Mountains in southern Colorado and descended the Rio Grande to Albuquerque, New Mexico, and it was on this expedition that Parry visited the region he designated as "Huefano Mountains."

The outstanding characteristic of the genus is the position of the oil tubes which are scattered throughout the pericarp as illustrated in the accompanying microphotograph (pl. 33) of a microtome section of the fruit. The simply pinnate leaves and conspicuous reflexed umbel rays are outstanding characters more easily noted.

2. Eryngium Wolffii, New Name

Eryngium Wolffii, new name

E. mexicanum Wats. acc. to Wolff, Pflanzenreich **61**: 178. *fig. 30.* 1913, non *E. mexicanum* Wats. Proc. Am. Acad. **26**: 136. 1891.

Herbaceous perennial, about 3 cm. high; roots many-fibrous; stems several, slender, suberect, trifurcately branched above; leaves numerous, sheathing at the base, lanceolate to oblong-ovate in general outline, 1–1.5 cm. long, deeply and irregularly pinnatisect, lobes linear-lanceolate, entire or irregularly dentate; inflorescence terminal on slender peduncles 1–10 cm. long, heads oblong-ovate, 1–1.5 cm. long, terminated by a linear, foliaceous appendage, entire or cleft at the apex; about 10 linear-lanceolate, entire, rigid, involucral bracts; fruit ovoid, about 2 mm. long, subterete in cross-section, papillose-echinate.

Type specimen: *Pringle 3180*, shallow ponds, Flor de Maria, State of Mexico, 1 Aug. 1890 (TYPE in the Gray Herbarium of Harvard University; cotypes in the Missouri Botanical Garden Herbarium, the Herbarium of the Academy of Natural Sciences, Philadelphia, the University of Chicago Herbarium, the Herbarium of the Field Museum of Natural History, the Herbarium of Carnegie Institute, Pittsburgh).

Distribution: Mexico in the states of Mexico and Morelos.

Specimens examined: shallow ponds, Flor de Maria, Mexico, 1 Aug. 1890, *Pringle 3180* (Gray Herb. TYPE, Mo. Bot. Gard. Herb., Phila. Acad. Herb., Univ. Chicago Herb., Field Museum Herb., Herb. Carnegie Inst.); Cerro de los Valgos, Morelia, alt. 2300 m., 1907, *Arsène* (U. S. Nat. Herb. 1157098).

Eryngium mexicanum as described by Watson[1] was based on a collection made by C. G. Pringle at Del Rio, State of Mexico, August 30, 1890, namely, No. 3229. Upon critical examination of cotype material of this number, it has been identified as *E. phyteumae* (*phyteumatos*) Delar.[2] The species, *E. mexicanum*, as described by Watson,[1] therefore goes to synonymy under *E. phyteumae* Delar. This is the position given the species by

[1] Watson, Proc. Am. Acad. **26**: 136. 1891.
[2] Delar. Eryng. Hist. 51. *pl. 21.* 1808.

Wolff[1] in his treatment of the genus *Eryngium* for the 'Pflanzenreich.' However, Wolff[2] retains the specific name, *E. mexicanum* Wats., for the plant collected by Pringle at Flor de Maria, State of Mexico, August 1, 1890, namely No. 3180. The latter plant has been found to be specifically distinct from *E. mexicanum*, as originally described, and, since that name has fallen to synonymy, must be given a new name. The name *Wolffii* is proposed for the species in honor of Hermann Wolff.

3. A Pimpinella New to North America

The common pimpernel, *Pimpinella Saxifraga* L., a native of Europe, has been introduced into waste places in eastern North America, becoming well established in certain localities. This form of the species has in all cases been recorded as glabrous, but a few specimens show a slight tendency toward a hirsute-pubescent condition.

During the past summer Dr. Harold St. John sent the author for determination a plant collected by Professor Morton E. Peck in a "meadow, 3½ miles s. e. of Roche Harbor, San Juan Islands, Washington, July 24, 1923," namely, *No. 13099*. Upon critical examination this plant proved to be *Pimpinella Saxifraga* L. subspecies *nigra* (Mill.) Gaud., as defined in the most recent survey of the genus by Wolff[3] for the 'Pflanzenreich.'

This subspecies is clearly distinguished by its conspicuous hirsute pubescence from the common "glabrous" *P. Saxifraga* previously reported in North America. The plant is of special interest not only as a new introduction into this country, but as the first record of the genus *Pimpinella* becoming established on the west coast or in western North America.

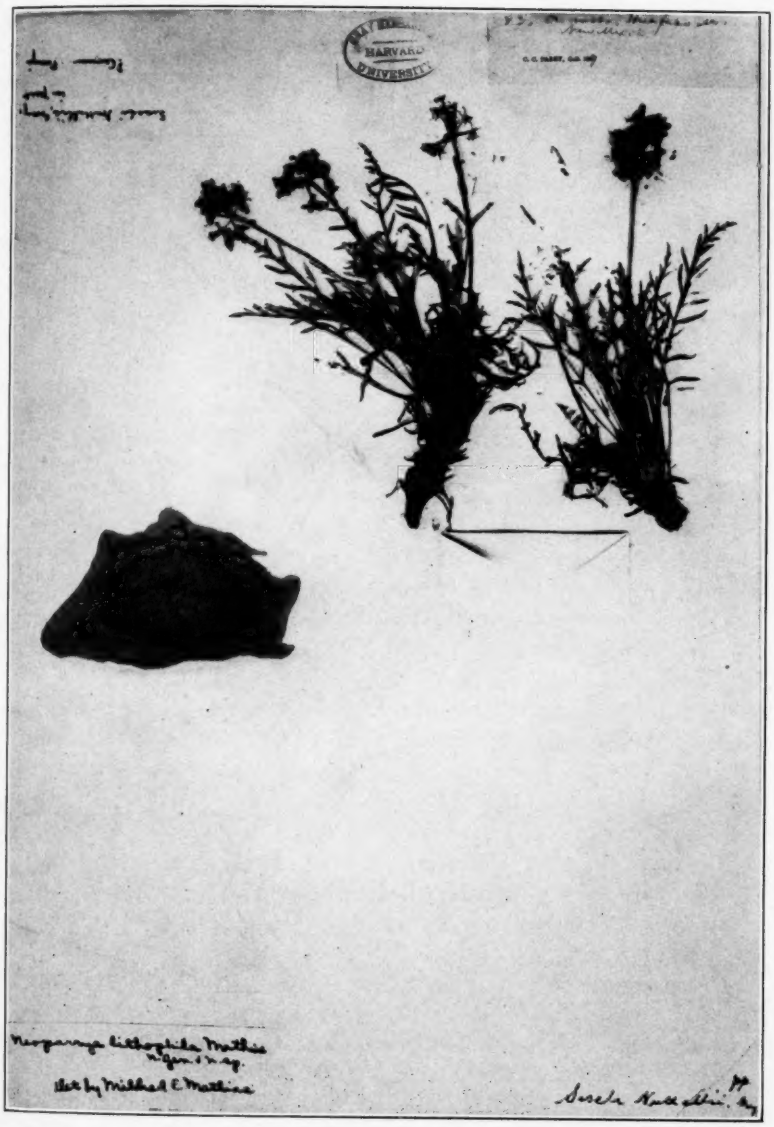
[1] Wolff, Pflanzenreich **61**: 188. 1913.
[2] Wolff, *Ibid* **61**: 178. 1913.
[3] Wolff, Pflanzenreich **90**: 302. 1927.

Explanation of Plate

PLATE 33

Neoparrya lithophila Mathias, from the type specimen, *Parry 83*, in the Gray Herbarium of Harvard University.

Superimposed on the type sheet is a microphotograph of a cross-section in the median plane of a mature fruit, taken from an isotype in the Missouri Botanical Garden Herbarium. × 25.



MATHIAS—STUDIES IN THE UMBELLIFERAE. II

NOTES ON SOUTHWESTERN PLANTS

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1. GAURA SUFFULTA ENGELMANN

In a collection of plants made in Oklahoma by Robert Stratton was a specimen of *Gaura* which could not be placed in any species known to occur in that state. After a critical survey of the genus and a comparison with type material it was found to be conspecific with *G. suffulta* Engelm.[1] This species has been known previously only from southern Texas, and its distribution as given by Small[2] is "On prairies or mountain slopes, Texas and northern Mexico," and by Coulter[3] "From the Colorado to the Lower Rio Grande, west to the Pecos and New Mexico." The accompanying map (fig. 1) shows the range of distribution of the species as represented in the Herbarium of the Missouri Botanical Garden, and gives the northern extension of the range as indicated by the Stratton specimen from the Arbuckle Mountains of southern Oklahoma, the Emig collections from the same region, and the Palmer and Glatfelter collections from Fort Worth and Dallas.

This species as described by Engelmann is quite distinct in its characters but has been poorly defined in subsequent works. Consequently a great amount of southwestern material of the genus *Gaura* has been erroneously referred to *G. suffulta*. The work of Wooton and Standley[4] in 1913 in segregating several new species from this complex has partially reinstated the species to the status originally given it by Engelmann. Its outstanding characteristics are a sessile, glabrous, oblong fruit somewhat constricted toward the apex, glabrous buds and caducous bracts prominently ciliate on the margins. The following material in

[1] Engelm. in Gray, Bost. Jour. Nat. Hist. 6 [Pl. Lindh. 2]: 190. 1850.
[2] Small, Fl. Southeastern U. S. ed. 2. 848. 1913.
[3] Coulter, Contr. U. S. Nat. Herb. 2 [Bot. West. Texas]: 118. 1891.
[4] Wooton and Standley, Contr. U. S. Nat. Herb. 16: 152–154. 1913.

Issued December 30, 1929.

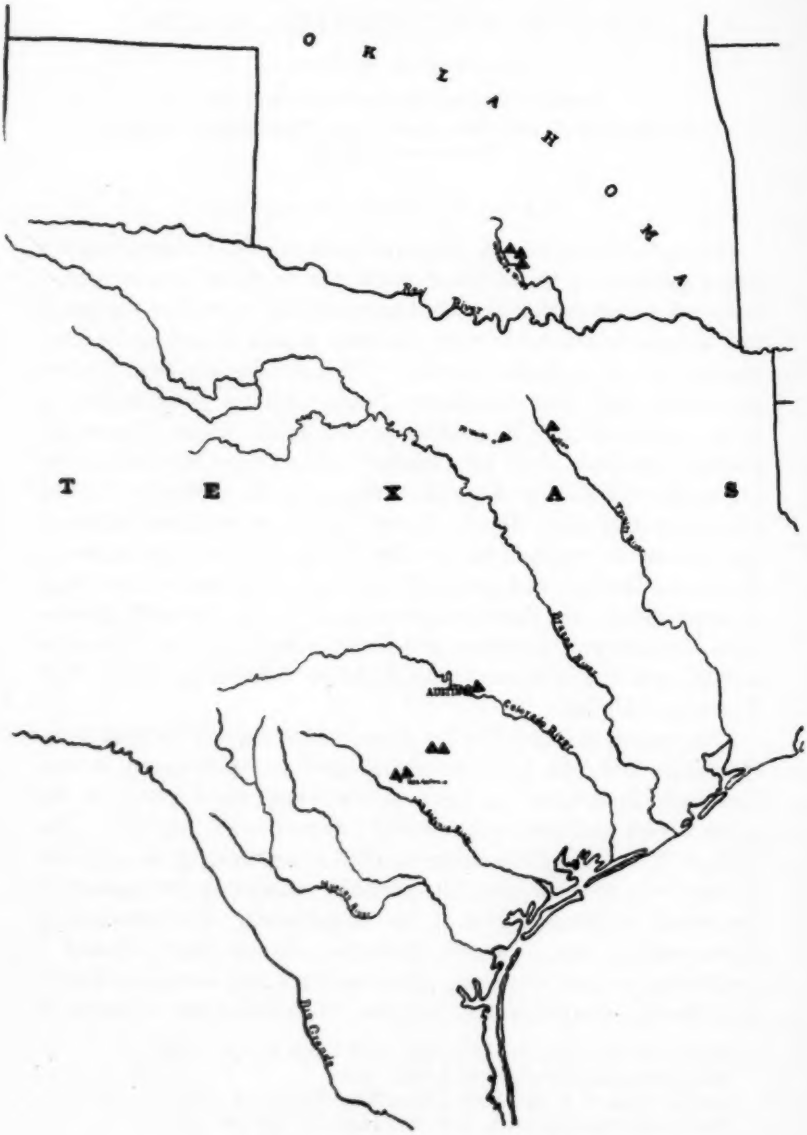

Fig. 1. Distribution of *Gaura suffulta*.

the collections of the Missouri Botanical Garden Herbarium has been found referable to the species as defined by Engelmann:

OKLAHOMA: rocky soil on mountain top, Price's Falls, Murray Co., 30 April, 1926, *Stratton 28;* Arbuckle Mts., Crusher, 12 May, 1916, *Emig 574;* Arbuckle Mts., near Davis, 21 June, 1917, *Emig 829.*

TEXAS: New Braunfels, May–June, 1847–8, *Lindheimer 611* (TYPE); New Braunfels, April, 1851, *Lindheimer 805;* dry hills, Austin, 9 May, 1872, *Hall 212;* rocky limestone hillsides, Ft. Worth, Tarrant Co., 24 May, 1918, *Palmer 3670;* San Antonio, *Wilkinson 73, 74;* tropical life zone, San Antonio, Bexar Co., 19 April, 1911, *Clemens & Clemens 697;* Dallas, 16 June, 1898, *Glatfelter.*

II. HOUSTONIA CROFTIAE BRITTON & RUSBY

Houstonia Croftiae Britton & Rusby, Trans. N. Y. Acad. Sci. **7**: 10. 1887; Coulter, Contr. U. S. Nat. Herb. **2**: 159. 1892; Greenman, Proc. Am. Acad. **32**: 284. 1897; Small, Fl. Southeastern U. S. 1108. 1903, and ed. 2. 1913.

Copious material of this species was found growing in the sand in the vicinity of Laredo, Texas, by J. Reverchon, March 21, 1903. The specimens had been referred doubtfully to *Houstonia humifusa* Gray. Upon critical examination the material was found not to represent *Houstonia humifusa* as defined by Gray and was determined from characters as *H. Croftiae.* As no authentic material of this species was available for comparison the specimen was sent to Dr. N. L. Britton, who verified the identification as *H. Croftiae* and stated that "It appears to agree exactly with my type specimen except in being a better developed and larger individual." This is a new station for this apparently rare species and seems to indicate a range for the species through southeastern Texas. The variation in size from the type material is so great that it seems worthy to mention such variations as occur by giving the following amplified description.

Houstonia Croftiae Britton & Rusby Plate 34, figs. 1–2.

A depressed-spreading annual; stems 1.5–10 cm. long, hirtellous with many scattered hairs; leaves 2–20 mm. long, oblanceolate, revolute, obtuse; stipules scarious, laciniate-dentate; flowers minute,

about 2 mm. long, sessile in the axils; calyx hirsute-pubescent; sepals linear-lanceolate; capsule subdidymous, 3 mm. high, about one-fourth inferior, hirsute-pubescent; seeds open-crateriform with a short hilar ridge.—Laredo, Texas, 21 March, 1903, *J. Reverchon 3951* (Mo. Bot. Gard. Herb.); San Diego, Duval Co., Texas, 1885, *Mary B. Croft 85* (N. Y. Bot. Gard. Herb., TYPE).

The type of the species shows these variations in stem and leaf length: the stems are from 0.4 to 2.5 cm. long and the leaves from 3 to 10 mm. in length as contrasted with the Reverchon material which has stems from 1.5 to 10 cm. long and leaves 2 to 20 mm. long; the flower and capsule sizes are approximately the same in both specimens.

This species is closely related to *H. humifusa* Gray and to *H. parviflora* Holzinger. Some of the most striking differences are shown in capsule and seed characters. *Houstonia parviflora* has a comparatively large capsule (pl. 34, fig. 3) about as broad as long, approximately three-fourths inferior and entirely glabrous. The calyx lobes are glabrous, short, and somewhat subulate. The seed is oblong-crateriform without a hilar ridge. *Houstonia Croftiae* has a smaller capsule (pl. 34, fig. 2), broader than long, less than one-fourth inferior and hirsute-pubescent. The calyx lobes are short and very hirsute. The seed is round, open-crateriform with a short hilar ridge. *Houstonia humifusa* has a comparatively small fruit (pl. 34, fig. 4), about one-fourth inferior and slightly roughened. The calyx lobes are linear-lanceolate, conspicuous, and usually recurved in the mature condition. The seed is oblong, somewhat concave on one surface with a hilar ridge extending throughout.

Explanation of Plate

PLATE 34

Fig. 1. *Houstonia Croftiae* Britton & Rusby, from a specimen collected by *Reverchon 3951*, in the Missouri Botanical Garden Herbarium.

Fig. 2. Mature fruit of *Houstonia Croftiae* Britton & Rusby, collected at Laredo, Texas, *Reverchon 3951*, 21 March 1903 (Mo. Bot. Gard. Herb.). × 7.

Fig. 3. Mature fruit of *Houstonia parviflora* Holzinger, collected at Vernon, Texas, *Reverchon*, 10 July 1903 (Mo. Bot. Gard. Herb.). × 7.

Fig. 4. Mature fruit of *Houstonia humifusa* Gray, collected at Raleigh, Texas, *Reverchon*, 15 April 1903 (Mo. Bot. Gard. Herb.). × 7.

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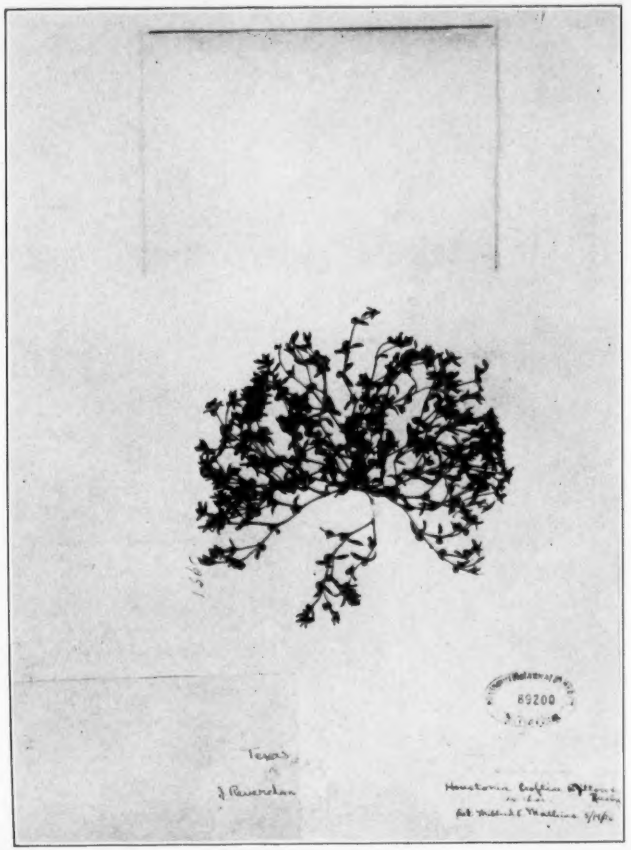

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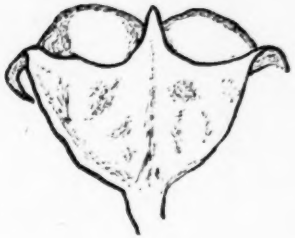

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4

A NEW VARIETY OF SENECIO AUREUS L.[1]

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About two years ago Mr. W. W. Ashe submitted to the writer for determination specimens of a plant which he had found in the mountainous region of western Virginia and eastern Tennessee. The plant in question is similar in habit and in all essential morphological characters to *Senecio aureus* L., a relatively common and well-known species of northeastern United States and adjacent Canada, but differs in having the lower surface of the leaves, especially those of the offshoots, densely white-tomentose in the early stages and only slightly glabrate in age. Furthermore, the leaves in the specimens from Mr. Ashe are somewhat thicker and firmer in texture and tend more strongly to an oblong-ovate outline than in typical *Senecio aureus* L. So far as known, this new variety has been found only in the valleys of the Middle and South Forks of the Holston River in western Virginia and eastern Tennessee, where it has been observed and collected by Mr. Ashe at a half dozen or more stations.

The origin of the plant is not known. It may be a mutant or, on the other hand, it may be a hybrid. There are some indications that it may be a hybrid between *Senecio aureus* L. and *Senecio tomentosus* Michx. The former of these two species occurs in the valley of the South Fork of the Holston River, and the latter species is relatively common in eastern Virginia. While the habit of the new plant is like *Senecio aureus*, yet the prevailing outline of the leaves of the offshoots and those of the lower parts of the upright stem, as well as their texture and tomentose character, suggests *Senecio tomentosus*.

The plant under consideration can scarcely be regarded as worthy of specific rank; and for the present, pending experimental evidence, it is deemed advisable to designate it as a new variety. Attention was called to this interesting case of variation at the meeting of the Botanical Society of America in Nashville in 1927, with the hope that additional material might be secured and its

[1] Issued December 30, 1929.

geographical range established, but no further information has been obtained. The writer takes pleasure in naming this rather remarkable variety for Mr. W. W. Ashe, who has made many important contributions to the knowledge of our flora. A description and record of distribution may be given, as follows:

Senecio aureus L. var. **Ashei** Greenman, n. var. Plate 35.

Caulis erectus 5-12 dm. altus; foliis inferioribus longe petiolatis, oblongo-ovatis, 3–10 cm. longis, 2–7 cm. latis, crenato-serratis juventate utrinque albo-floccoso-tomentosis supra denique plus minusve glabratis.—VIRGINIA: moist meadows, near Marion, Smyth County, May 26, 1927, *Ashe*, TYPE; near Atkins, Smyth County, May 27, 1926, *Ashe;* moist meadows, limestone valley soil, near Lodi, Washington County, May 26, 1926, *Ashe*. TENNESSEE: moist meadows, near Johnson City, Washington County, May 24, 1926, *Ashe*.

The type specimen is in the private herbarium of Mr. W. W. Ashe, Washington, D. C. A portion of the type and photographs are in the Missouri Botanical Garden Herbarium.

EXPLANATION OF PLATE

PLATE 35

Senecio aureus L. var. *Ashei* Greenman, n. var.

From the type specimen in the herbarium of Mr. W. W. Ashe.

GREENMAN—SENECIO

STUDIES IN THE APOCYNACEAE. IIIA

A New Species of Amsonia from the South-Central States

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While engaged in the preparation of a recently published monograph of the genus *Amsonia*,[1] the writer was conscious of an element not quite typical in the exsiccatae which he referred to *A. Tabernaemontana* L. var. *Gattingeri* Woodson. The anomalous specimens were characterized superficially by a pubescent calyx and more elongate, shining foliage than the typical material. The plants appeared indigenous only to Missouri, southeastern Kansas, eastern Oklahoma, and northeastern Texas. Unable to perceive unquestionable evidence of individuality, however, the specimens were referred to the above variety until the acquisition of additional data.

During a collecting expedition in the autumn of 1928, the author encountered a large colony of these anomalous Amsonias near Bourbon, Missouri. Again in the spring of 1929 they were discovered near Hines and Gainesville, also in Missouri, the latter only a few miles north of the Arkansas state line. From these, as well as other observations in the field, the specific individuality of the plants appears quite evident. A description follows:

Amsonia illustris Woodson, n. sp.[2]

[1] Woodson, R. E., Jr. Studies in the Apocynaceae. III. Ann. Mo. Bot. Gard. **15**: 379–434. *figs. 1–3, pl. 50–53.* 1928.

[2] **Amsonia illustris** sp. nov., herbaceis perennis; caule erecto ramoso striato omino glabro 7–11 dm. alto; ramis erectis vel pauce ascendentibus; foliis alternis vel subverticillatis petiolatis membranaceis lanceolatis vel lineari-lanceolatis 5–7 cm. longis 1.0–1.5 cm. latis supra coriaceis illustris subtus glabris; lobis calycis hirsuto-strigillosis 1.5–3.0 mm. longis, triangulo-oblongolanceolatis; corolla salverforma puberula, tubo 6–8 mm. longo, limbo 5-partito 4–6 mm. lato; stigmate apice truncato; folliculis teretibus gracilibus continuis vel subtorulosis glabris sessilibus 8–14 cm. longis.—Missouri: collected along beds of gravelly branches near Webb City, July 15, 1909, *E. J. Palmer 2438* (Mo. Bot. Gard. Herb. type).

Issued December 30, 1929.

Herbaceous perennial from a somewhat thickened, woody rootstalk; stems terete, 7–11 dm. tall, 5–10 mm. in diameter at the base, 2–3 mm. in diameter at the inflorescence, glabrous, clustered from the base, erect, bearing scale-like cataphylls towards the base, branched above, the branches ascending, nearly erect, about the length of the main stem; leaves membranaceous, alternate or somewhat whorled below, numerous, ascending or spreading, lanceolate to linear-lanceolate, acuminate at both base and apex, narrowing almost imperceptibly to the short petiole, the blades 5–7 cm. long, 1.0–1.5 cm. broad, glabrous or somewhat glaucous beneath, coriaceous and shining above; inflorescence relatively large and dense, barely held above the foliage, pedicels .25–.75 cm. long; calyx 1.5–3.0 mm. long, hirsute-strigillose, the lobes triangular oblong-lanceolate; corolla salverform, caerulean blue shading to buff at the base of the tube, the tube 6–8 mm. long, 2–3 mm. in diameter, pubescent without, the lobes spreading or slightly ascending, oblong to oblong-lanceolate, acuminate at the apex, 4–6 mm. long; stigmatic-cap about as long as broad, stigma depressed-capitate; follicles continuous or subtorulose, 8–14 cm. long, 2–3 mm. in diameter, gradually acuminate, sessile, glabrous, 9–30-seeded; seeds 5–11 mm. long, truncate-oblong, variously pitted and wrinkled, cinnamon-brown.

Distribution: sand-bars and gravelly banks of streams, occasionally spreading into fields, Missouri, southeastern Kansas, eastern Oklahoma, and northeastern Texas.

Specimens examined:

Missouri: Moselle, July 2, 1886, *Eggert* (MBG); Webb City, gravelly branches, June 25, 1909, *E. J. Palmer 2339* (MBG); same locality, Sept. 2, 1909, *E. J. Palmer 2620A* (MBG, G); Gray's Summit, May 15, 1926, *Greenman 4493* (MBG); banks of the Meramec River, Minke, St. Louis Co., May 17, 1919, *Greenman 3944* (MBG); Webb City, common in woods, May 3, 1902, *E. J. Palmer 296* (MBG); same locality, common along beds of gravelly branches, July 15, 1909, *E. J. Palmer 2438* (MBG); Allenton, date lacking, *Letterman* (MBG); Spring Park, May 24, 1892, *Dewart* (MBG); Valley Park, St. Louis Co., May 15, 1897, *Trelease 459* (MBG); Franklin Co., July 2, 1886, *Eggert* (MBG); Carter Co., rocky banks, May 1, 1891, *Bush 966* (MBG); Carter

Co., May 1, 1891, *Mann* (MBG); Gascondy, July 21, 1914, *Emig 221* (MBG); Elmont, May 23, 1914, *Emig 250* (MBG); Jerome, June, 1913, *Kellogg 116* (MBG); Fox Creek, Allenton, June, 1880, *Letterman* (MBG, US); low ground, only two plants, south of Lee's Summit, June 3, 1917, *Hoffman* (MBG); chert barrens, Jasper Co., July 12, 1927, *Kellogg 1107* (MBG).

KANSAS: rocky soil, Allen Co., 1896, *Hitchcock* (MBG).

OKLAHOMA: on dry bank of draw, near Miami, Ottawa Co., Aug. 26, 1913, *Stevens 2337* (MBG, G, US).

TEXAS: swamps and low prairies, near Dallas, April 21, 1902, *Reverchon 3123* (MBG, G); Long Lake, Anderson Co., June 9, 1899, *Eggert* (MBG).

The abbreviations employed in the foregoing section refer as follows: MBG—Herbarium of the Missouri Botanical Garden; G—Gray Herbarium of Harvard University; US—United States National Herbarium.

Amsonia illustris constitutes a distinct addition to the subgenus Euamsonia by reason of several characteristics. It is the only species in the section with pubescent corollas which displays a hirsute calyx except *A. ludoviciana*, from which it differs in the texture of the leaves and follicles, geographical distribution, and additional particulars. The coriaceous and shining foliage, from which the species derives its name, is somewhat reminiscent of that of *Nerium Oleander* in shape and texture.

The follicles are unusually long, almost papery in texture, and are lax, sometimes even pendulous in position, an anomaly for the entire genus. The character that is most striking technically, however, is the construction of the follicles which are subtorulose: somewhat constricted between the seminiferous regions and inclined to dehisce immediately after maturity by breaking into several transverse sections. This character, however, is merely a suggestion of the condition of the follicles in the subgenus Articularia, and the follicles of *A. illustris* are in all important particulars typical for the subgenus Euamsonia. An additional anomaly of the follicles of the species is a tendency found in several specimens for only one carpel to develop, forming a single unusually stout and long follicle and bearing the sterile carpel as an inconspicuous basal protuberance.

Since the follicles of *A. illustris* are absolutely glabrous and otherwise unlike those of *A. ludoviciana*, its most pronounced affinity is with *A. Tabernaemontana* and its varieties, from which, however, it is readily separable by its hirsute calyx, its strikingly coriaceous, shining foliage, its scale-like cataphylls, which in the latter species are semifoliaceous, and its lax, subtorulose follicles.

A. illustris is a much more ornamental plant horticulturally than its relative *A. Tabernaemontana*, with which it has been cultivated in the author's garden for two seasons. Not only are the compact inflorescences more floriferous, but the habit of the plant itself is much more robust and compact, and the beautiful oleander-like foliage is almost evergreen. The foliage of the species is also somewhat reminiscent of that of the willow, which has occasioned the frequent identification by collectors as *A. salicifolia* Pursh (*A. Tabernaemontana* L. var. *salicifolia* (Pursh) Woodson).

PRELIMINARY STUDIES IN THE GENUS DALDINIA[1]

MARION CHILD

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INTRODUCTION

The problem of fungous spore germination has not infrequently been the subject of physiological investigation, but many of the investigations have been carried on in the lower groups of fungi where the spores germinate relatively easily in comparison with those of the Ascomycetes, where germination is physiologically much more complex. It is only with a great deal of difficulty and after many types of experiments that germination is secured.

According to Saccardo ('82) and Ellis and Everhart ('92) there are in North America only four known species of *Daldinia*.

[1] An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of Master of Science in the Henry Shaw School of Botany of Washington University.

These are *D. concentrica* (Bolt.) Ces. & De Not., *D. vernicosa* (Schw.) Ces. & De Not., *D. cingulata* (Lév.), and *D. loculata* (Lév.). Ellis and Everhart are of the opinion that *D. cingulata* is doubtfully distinct from *D. vernicosa*, but it seems to the writer that neither the descriptions of *D. cingulata* nor *D. loculata* are adequate. In this paper, however, the taxonomy of the group as a whole is not considered; the primary interest is a study of the factors influencing the germination of the ascospores and the growth of the mycelium of *D. concentrica*, *D. vernicosa*, and two undescribed species designated as *Daldinia X* and *Daldinia Y*.

As far as the writer is able to learn, a thorough study of ascospore germination of *Daldinia* has never been undertaken. Since the species *D. concentrica* is so widely distributed its ascospores have been observed and have been germinated in order that the life history of the fungus might be completely known. Its ascospores germinate rather readily in comparison with those of *D. vernicosa*. The ready germination of the ascospores of *D. concentrica* probably explains the fact that several workers have studied the fungus in pure culture. These workers have not, however, studied the influence of more than very ordinary factors on ascospore germination.

In striking contrast to the ascospores of *D. concentrica*, which germinate readily, are those of *D. vernicosa* which germinate only under very special conditions. Just as these two species are quite distinct morphologically, they are even more different physiologically.

In addition to the preceding species there are two as yet undescribed, *Daldinia X* and *Daldinia Y*. The former was collected in two different localities in Missouri and could not be classified among the described species of North America or even of foreign countries. It is, however, more closely allied to *D. vernicosa* than to *D. concentrica*. It differs, however, from *D. vernicosa* in size and color of ascocarp and ascospores. *Daldinia Y*, a species from the Black Hills of South Dakota, apparently is more closely related to *D. concentrica* and was so named in the herbarium of the Missouri Botanical Garden. It differs from the typical species by size and markings of the ascocarp, and in the size and color of the ascospore. In both of these undescribed

species the morphological differences are completely correlated with physiological ones.

In view of the fact that the distribution of *D. concentrica* is so universal and also since so many forms have been included in the species, it seemed desirable to the writer to study this species not only from the morphological but from the physiological point of view, for environmental conditions may so completely modify a species that uncritical investigators may be led to the wholesale creation of new species. It seemed fitting that a more modern attitude should be taken and that morphological characters should be correlated with physiological characters as shown by pure culture. By adopting such an attitude, it is possible not only to obtain a clearer conception of a species but also to determine its biological reactions.

Further investigations concerning the cytology and taxonomy of the ascigerous stage of North American material are under way.

History

There are, as far as the writer is able to learn, only eight investigators who have published studies of *Daldinia*. Only three of these investigations were published as separate and special studies, the others merely being a result of work done in a large group of which *D. concentrica* was a member. *D. concentrica* with one exception was the only *Daldinia* studied in pure culture. The majority of investigators merely observed the ascocarp, ascospores, and conidia, in the natural habitat.

Among the first investigators were the Tulasnes ('63), who published a description of the ascocarp and also noted in the natural habitat the presence of conidia on the ascocarp previous to the formation of perithecia. Twenty-eight years later Brefeld ('91) described the ascocarp in its natural habitat and also observed ascospore germination.

As part of an investigation of the fungi of Brazil, Möller ('01) made observations on the biology of the fungus. He observed the rapidity of the growth of the stroma and the duration of ascospore production in the natural habitat. From the germinated ascospores he obtained the conidial stage in culture. Information as to media and methods is insufficient.

The first worker to make a special study of *D. concentrica* was Molliard ('04), who studied particularly the conidial stage in order to assign it a definite taxonomic position and name. Germination of the ascospores was obtained after three days at 15° C. on slices of carrot, and on other media which were not stated. In observing the development of the mycelium, changes in color and the development of nodular tufts were noted, the nodular tufts being reminiscent of the stroma of the perfect stage. Conidia were at first obtained after two months, but in later experiments were observed to appear within two or three weeks. They were noted to be ovoid, almost colorless, 7–8 μ long × 4.5–5.4 μ wide, and pointed at the end where they were attached to the little sterigmata-like base. For the conidial stage he proposed the name *Nodulisporium Tulasnei*.

A few years later Brooks ('13) published the results of some experiments with the Ascomycetes and Basidiomycetes. In this paper there is reference to his obtaining from germinated ascospores of *D. concentrica*, conidia in pure culture. *Fraxinus* wood blocks sterilized in tubes, the bottoms of which held plugs of water-saturated cotton were used as a medium. The cultures were placed in dull light at room temperature, and the resulting mycelium, which was at first white, became tawny with age and where coming in contact with the glass became blackened, aggregated so that it resembled the hyphae which formed the matrix of the stroma. The conidiophores were branched and from them elliptical hyaline spores, 6–8 × 3–4 μ, borne singly and in heads, were abstricted. The hyphae readily penetrated the wood and were especially abundant in the vessels.

One of the more recent workers on *Daldinia* is Elliot ('20) who studied the formation of conidia and the growth of the stroma. With conidia from the natural habitat *Fraxinus* blocks were inoculated, then placed in the open on the ground. *Fraxinus* chips were inoculated and kept in the laboratory. Although she did not clearly distinguish between results with pure culture and those in open air, it is evident that most of the observations were made on the material in the open air. Ascospores germinated on the exterior of the ascocarp and in the perithecia when kept in a damp condition under a bell jar, and the mycelium thus pro-

duced gave rise to conidia in a few days. The conidial form under natural conditions appeared as a cream-colored incrustation of conidiophores that were much branched in a verticillate manner and terminated in clusters of hyaline conidia. For the most part the discussion is given over to the perfect stage.

Following the investigations of Miss Elliot are those of Miller ('28), who has studied the biology of the *Sphaeriales* and in this group has included some investigations on *D. concentrica*, *D. Escholzii*, *D. vernicosa*, and *Hypoxolon placentiforme*, noting that in all forms the conidial layer is a light grayish-brown, that the conidia are hyaline with a slight greenish tint, are 6–8 μ long × 4–5 μ wide, and are borne on sympodially branched conidiophores. No work was done on spore germination.

The last paper concerning *Daldinia concentrica* has been published by Panisset ('29), who identified this fungus as the one which commonly attacks *Fraxinus excelsior* and causes an irregular brown to black coloring known to timber merchants as "calico wood." In order to identify this fungus she germinated the ascospores on various solid and liquid media, observed mycelia and the discoloration of the medium resulting from the germination of the conidium, finding that the discoloration agreed with that resulting from the germination of the ascospores and with the hyphae and brown staining in the calico wood. The external conditions of growth as regards water, oxygen acidity and alkalinity, the food materials utilized by the fungus inside the wood, and the nature and production of the brown substance were investigated.

Materials and Methods

These studies were chiefly with pure cultures isolated from collections made by the writer in Pulaski County, Missouri; by Dr. Linder in Missouri, and Canton, Massachusetts; and with material from the herbarium of the Missouri Botanical Garden.

In all except a few cases ascospore germination was studied in hanging-drop or van Tieghem-cell cultures. The ascospores, directly from the freshly cut perithecia, were placed on the medium by means of a sterile platinum needle. Unless otherwise indicated, the ascospore cultures were always put into a dark

cabinet of approximately room temperature, for better germination was secured in the dark than in the light. When a liquid medium was used as a source of nutrition, three or four drops of the same solution were placed in the bottom of the cell in order to insure equilibrium within the cell and thus to prevent changes in the concentration of the solution. When a solid medium was used the nutrient solution minus the agar was placed in the bottom of the cell. This also served as a source of the necessary moisture. All cultures both in the study of spore germination and mycelial growth were made in duplicates or triplicates; frequently erratic results necessitated repetition as many as ten times. Spore counts were made under low and high power of the microscope. Two hundred ascospores from different fields were counted in each Van Tieghem cell and then an average taken. The tables not only include the results of the individual experiments, but the averages of them. This was done in order to show the occasional erratic behavior of the fungus. The graphs are based upon the average of a stated number of experiments which will be indicated in each case. In both the tables and the graphs the discussion is based upon the average.

The isolation of the fungi for experiments on mycelial growth was made by the single-spore method of Kauffman, with one exception, *Daldinia Y* (Black Hills, South Dakota), in which case the material was twenty-five years old and the ascospores would not germinate under a variety of conditions. In this case the isolation was made by washing the surface of the ascocarp with a 70 per cent solution of ethyl alcohol, then with a sterile knife a piece of the stromatic material was cut out and dropped into a few cc. of sterile distilled water to which had been added a few drops of 10 per cent lactic acid. After a thorough shaking, the material was put into an abrasion on the agar plate.

With few exceptions all of the observations made on mycelial growth were from cultures in petri dishes, which had been inoculated with ¾–1 sq. cm. of actively growing material of the same age. The radial growth was measured from the periphery of the inoculum to the periphery of the colony; the first measurement was made two days after the plates were inoculated and the second measurement a few days later, before the mycelium had

covered the surface of the medium. The rate of growth per day was then calculated by subtracting the first measurement from the second and dividing by the number of days which intervened. This was done in order to obtain more accurately the rate of growth per day, thus averaging rapid growth which might occur due to mycelium stored with food products, and growth which was slight at first due to relatively little stored food products.

For germination, both solid and liquid media were used, but for the study of mycelial growth, solid media only. Among the latter are oatmeal, Leonian, Pfeffer (Fe omitted), prune, *Tupelo* sp., and *Platanus occidentalis* L. decoction agars, prepared according to the regular formulae. For hydrogen-ion work, Pfeffer's solution as prepared by Gillespie ('18) with the addition of 3 per cent agar was used; the solutions were made acid by citric acid and basic by NaOH. They were tested by the colorimetric method before and after sterilization.

Experimental Data

Experiments on the Germination of Old Ascospores

In these experiments herbarium material from the Missouri Botanical Garden and fresh material from other sources were used. The herbarium material included three different collections of *D. concentrica* from Missouri, one from the Black Hills of South Dakota, one from Alabama, and two from the Philippine Islands, and one, *D. vernicosa*, from Missouri. The fresh material used in the latter part of this study included *D. concentrica*, *D. vernicosa*, and *Daldinia X* from Missouri. One *D. vernicosa* came from Canton, Massachusetts.

The names of the fungi, the places where they were collected, and the age will hereafter be abbreviated as follows:

Herbarium material:

D. concentrica Philippine Isl. 16 yrs. old—*D. concentrica* (No. 12465)

D. concentrica Philippine Isl. 12 yrs. old—*D.* concentrica (No. 59960)

D. concentrica Meramec, Mo. 23 yrs. old—*D. concentrica* (No. 16363)

D. concentrica Auburn, Ala. 3 yrs. old—*D. concentrica* (No. 16365)

D. concentrica St. Louis, Mo. 32 yrs. old—*D. concentrica* (No. 43049)

D. vernicosa Lesterville, Mo. 2 yrs. old—*D. vernicosa* (L. Mo.) (No. 63847)

D. vernicosa Canton, Mass. 3 yrs. old—*D. vernicosa* (C. Mass.)

Daldinia Y Black Hills, S. Dak. 25 yrs. old—*Daldinia Y* (B. S. D.) (No. 43121)

Fresh material:

D. concentrica Dixon, Mo.—*D. concentrica* (D. Mo.)

D. vernicosa Dixon, Mo.—*D. vernicosa* (D. Mo.)

Daldinia X Valley Park, Mo.—*Daldinia X* (V. P. Mo.)

At first various attempts were made to secure germination with two-year-old ascospores of *D. concentrica* (D. Mo.) but without success. Tap water and distilled water were of no value, and although Stevens ('98) found that ethyl and methyl alcohol in different normalities acted as specific stimuli to the spores of various fungi, this treatment also failed. By soaking the ascospores of *D. concentrica* (D. Mo. 2 yrs. old), *D. vernicosa* (L. Mo.), and *Daldinia Y* (B. S. D.) in ethyl alcohol for different lengths of time up to a week, then putting them into weak sucrose solutions, the spores merely swelled and did not germinate. The ascospores of *D. concentrica* (D. Mo.) and of *Daldinia Y* (B. S. D.) swelled more than those of *D. vernicosa* (L. Mo.) and *D. vernicosa* (C. Mass.). When weak sucrose solutions of 1–10 per cent seemed to induce swelling, additional percentages were tried. However, 20 per cent solution caused plasmolysis and no germination.

All of the ascospores were observed to have hyaline exospores, which in any of the fresher *D. concentrica* sloughed off under moist conditions, while in the older material they cracked off irregularly. The exospores were about .07 μ and were stained by the lacto-phenol-cotton-blue mounting medium. The apparently chitinized amber-colored wall which remained after the hyaline exospore sloughed off was very thin. The hyaline exospores of *D. vernicosa* were only about 105 μ thick and did not take the stain of cotton-blue in lacto-phenol as well as did those of *D. concentrica*. The chitinized amber-colored wall of the former was heavier than that of the latter species. In fresh material the exospore of *D. concentrica* under moist conditions sloughed off

about five hours after the culture was made up but soon disappeared by dissolving in the medium. In only a few cases have exospores of *D. vernicosa* been observed while sloughing off. The exospore ruptures irregularly, never regularly as in fresh material of *D. concentrica*, and probably also dissolves in the medium.

Some investigators have observed that the "spore pellicle" or exospore must be dissolved or cracked off before germination will occur. Hartig ('85) found that ammonia or some alkali was necessary for dissolving the "spore pellicles" of *Merulius lachrymans* before germination would occur. This does not seem to be necessary in *Daldinia*, but it does seem necessary to dissolve or break down the amber-colored chitinized spore wall. In attempting to rupture this wall, the ascospores were ground on a rough glass by means of a scalpel, then placed in a small quantity of sterile distilled water to which a few drops of a 10 per cent solution of lactic acid were added, or different percentages of alcohol or sucrose or wood decoctions. The hyaline exospores were cracked off irregularly by this mechanical means, but the walls of the spores were not affected, and no germination occurred.

Whether the spores needed a stimulus or a perfect source of nutrition, or both, had to be determined. Since the fresh material had not been fumigated, the spores might still be viable, and would germinate if the correct nutrient were found. Some standard agar medium, oatmeal, Pfeffer, Pfeffer with the addition of sucrose, string-bean, and prune decoctions, was tried. All of the above-mentioned herbarium material and material from other sources were used. The ascospores of *D. concentrica* (Nos. 16363, 16365, 43049) on all of the media showed irregularly cracked exospores and a slight swelling, but no germination after 120 hours, which time shall be taken as the limit although the cultures were run for two weeks. In the media where there was a high sugar content, swelling was in most cases more pronounced, always more so in *D. concentrica* than in *D. vernicosa*. The exospores of *D. concentrica* (Nos. 12465 and 59960) cracked from one apex to the other in a characteristic manner after 120 hours on prune-decoction and on Pfeffer-solution agar to which sucrose had been added. In some cases the hyaline exospore became roughened

but did not crack off. On other agar media the character of the spores did not change appreciably. When alternately shocked by dry freezing in an electric refrigerator at – 5° C. for two days, then heated in an incubator at 40° C. for the same length of time after which the ascospores were put on the different media, the exospores cracked from one apex to the other. Some ascospores on prune-decoction agar and Pfeffer-solution agar to which sucrose had been added cracked open longitudinally, due probably to the combination of heating, freezing, and high sugar content of the media. In no case did germination occur. When the ascospores were frozen they seemed to shrink, but upon heating regained their normal size.

The ascospores of *Daldinia Y* (B. S. D.) became considerably swollen and the oil globules became more pronounced in a 20 per cent sucrose solution, but plasmolyzed after 48 hours. On prune-decoction agar the exospores sloughed off irregularly and the oil globules also became larger. In some cases the ascospores cracked open, due probably to the high osmotic pressure which results from a medium of high sugar content.

Since *D. vernicosa* is frequently found on charred logs, it was suggested that heat might play some part in germination; therefore the spores were heated by putting them in a small vial which was partially immersed in a double boiler, in which the water was allowed to boil for a few minutes. The ascospores of *D. vernicosa* (No. 63847) were then put on the different media, but in no case did they germinate, although the spores were slightly swollen and the exospores roughened. Maneval ('26) studied eight different species of rust teliospores and found that physiologically the spores changed during the winter. A sort of "ripening" occurred, for as the spring approached the percentage of germination in a given species requiring a rest period increased gradually to a maximum and then declined. As the time of maximum germination approached the time necessary for germination to start decreased to an hour or less. Since overwintering is considered a factor of real significance in the germination of fungous spores, an artificial means of overwintering was employed here by freezing the spores in a refrigerator. The ascospores were put into small vials containing a few cc. of sterile

distilled water and frozen for a week at – 5° C. After that time the ice was melted and the ascospores were sprayed on a plate of oatmeal agar. Some of the ascospores cracked open, while others swelled slightly, but again there was no germination. Ascospores of *Daldinia Y* (B. S. D.) when treated alternately with freezing and heating did not swell; the exospores cracked off irregularly, but there was no germination. As in the preceding the exospores of *D. vernicosa* (C. Mass.) were observed to be cracked off after having been heated, but in the controls the exospores were in the majority of cases not even roughened. When on a medium with a high sugar content, the ascospores swelled considerably but by no means to the same extent as in *D. concentrica*. Other media gave no better results. Alternate dry freezing and heating caused a cracking open in most cases, but two or three ascospores of *D. vernicosa* (C. Mass.) germinated out of thousands that were in the cultures. These ascospores were kept under observation for a month, but for no apparent reason their development was soon arrested (pl. 37, fig. 4).

Although it is known that some fungous spores remain viable over a period of many years, even after fumigation, it is quite obvious from the results of these experiments that the viability of these ascospores is seriously affected either by age or disinfectant (in this case CS_2). Nevertheless in spite of negative results it is apparent that the ascospores of *D. vernicosa* are much more resistant than those of *D. concentrica* and its relative, *Daldinia Y* (B. S. D.). This has a direct bearing on the interpretation of the later results.

The preceding experiments show that water and alcohol have little effect on such old ascospores. Grinding merely cracked off the exospore and did not affect the chitinized wall. The older material showed a more irregular cracking off of the exospore than the fresher material. Certain concentrations of sucrose, prune-decoction agar, and Pfeffer-solution agar to which sucrose had been added caused plasmolysis and even a bursting open of the ascospore. Alternate dry freezing and heating might also have the same effect. Short periods of heating alone have little effect, but in one case freezing induced a very small percentage of germination of *D. vernicosa* (C. Mass.) ascospores.

EXPERIMENTS IN THE GERMINATION OF FRESH ASCOSPORES

In the experiments which follow, fresh material of *D. concentrica* (D. Mo.), *D. vernicosa* (D. Mo.), and *Daldinia X* (V. P. Mo.) only was used, since most of the results of the preceding experiments gained from old material were negative.

EFFECT OF OXYGEN

In attempting to determine one of several factors which evidently influence germination of ascospores, an excess of oxygen was tried in order to determine whether or not the oxygen supply was a factor in the germination of *Daldinia* ascospores. DeBary ('87) stated that oxygen for the germination of fungous spores had never been sufficiently demonstrated and that perhaps spores germinated only after the oxygen in the drop culture had been exhausted. There are investigators who have proved the necessity of oxygen for germination. Maneval ('26), for example, has found that for the germination of teliospores of the rusts the oxygen supply is a very important factor. Reed and Crabill ('15) found that oxygen was apparently necessary for the germination of *Gymnosporangium Juniperi-virginianae*, and that the carbon dioxide of the atmosphere prevented germination. That a scanty oxygen supply prevented the germination of the spores of *Plenodomus fuscomaculans*, Coons ('16) was able to demonstrate.

In the first oxygen experiment small amounts of the alga *Pleurococcus* sp., were put in the bottom of the van Tieghem cells containing distilled water. The cover glasses were only lightly put on the cells in order to allow some circulation of air. Then the cells were put in a window where the light was abundant. After two days, more water was added and the spore inoculations were made on Pfeffer agar. Slightly better germination of *D. concentrica* in cultures in which the alga was used was obtained than in the controls. After 96 hours 80 per cent germination was secured, while in the controls 78 per cent. The only other difference in germination was a more profuse branching of the mycelium in the cultures containing the alga than in the controls. The ascospores of *D. vernicosa* germinated slightly better in the controls than in the cultures where the alga was used. After

96 hours 61 per cent germination was secured, while in the controls 10 per cent. The ascospores in the cultures with the alga swelled more, became a lighter brown, and had longer germ-tubes than those in the controls. It would seem from these results that an abundance of oxygen accelerates growth of the germ-tubes in both cases, but does not increase the percentage of germination in *D. vernicosa*.

The second means of obtaining an extra supply of oxygen was a 3 per cent solution of hydrogen peroxide, which was used as a medium and as the liquid in the bottom of the cell; the slight trace of alkali in the glass and the light cause the decomposition of hydrogen peroxide with a release of oxygen. After 120 hours the ascospores of *D. concentrica* and of *D. vernicosa* in hydrogen peroxide had not germinated. After 24 hours the ascospores of *D. concentrica* had swollen slightly and had become a lighter brown. The ascospores of *D. vernicosa* had become considerably swollen and the natural brownish-black color which is so characteristic of mature *D. vernicosa* spores had become a light brown, due to an oxidizing process. Ordinarily the spores retain their dark brown color even after germination. Since no germination was secured when the spores were put into the hydrogen peroxide, Pfeffer agar was used as the medium with hydrogen peroxide in the bottom of the cell. No germination was obtained in *D. concentrica* where hydrogen peroxide was used, although in the controls it did occur. The ascospores of *D. vernicosa* in hydrogen peroxide germinated poorly; the spores although considerably swollen were evidently oxidized. The germ-tubes were short, very wide, and very granular. Germination however poor was slightly better in the controls, and the short germ-tubes were less granular.

Since these methods of supplying oxygen proved inaccurate, an oxygen tank was employed. From a small tank of 40-gallon capacity a slow stream of oxygen was allowed to pass into a large water-filled bell jar suspended in a cylinder of water. As the oxygen passed out into the bell jar the water passed into the cylinder and the bell jar rose. After considerable experimenting a steady stream from the tank balanced the stream out of the bell jar, but this stream could not be maintained for more than

six hours. By clamps on the tubing leading from the tank to the bell jar and from the bell jar to the first tower, the stream of oxygen was regulated. From the bell jar the oxygen went through a tower of KOH, then through a gas-washing bottle containing a saturated solution of $Ba(OH)_2$; thus there was a double check for the elimination of CO_2. On a wooden rack the height of the stage of the microscope a series of Wardian cells, the same size as the van Tieghem cells, was mounted on slides by means of balsam. These cells were supplied with glass inlets and outlets 6 cm. long and 3 mm. in diameter. The hanging-drop method was used with Pfeffer agar as the medium, then the cover glasses were cemented to the cells by carefully applying a mixture of beeswax and paraffin. Since this cement would not withstand the pressure of the flow of oxygen, weighing bottles containing about 10 cc. of mercury were put on top of the cells, after the beeswax-paraffin preparation had been applied. From the cells the oxygen passed into a shallow cylinder of water and the bubbles counted here were used as an indication of the number of bubbles per minute that passed through the cultures. All the connections were carefully sealed with shellac in order to insure a leak-proof system. The apparatus was set up in a dark-room at a temperature of approximately 24° C. To examine the cultures the cells were carefully moved onto the stage of the microscope. No detachment was necessary since the rack was open at both sides and the microscope light could be moved along freely with the microscope. After some experimenting it was found necessary to divert the flow of oxygen through Y-tubes then into the cells in order to secure a steady flow of oxygen. A constant flow of oxygen of 68 bubbles per minute for six hours was maintained with only six cells in two parallel rows connected by Y-tubes of 5 mm. in diameter.

The results of these tests indicate that ascospores of *D. concentrica* supplied with oxygen from the tank germinated slightly better than the ascospores in the controls. The ascospores treated with oxygen did not swell as much as those not treated, but their exospores sloughed off and disappeared sooner. The flow of oxygen may carry the ascospores away or they may be absorbed by the medium more quickly than when not treated

with oxygen. In almost all cases, the germ-tubes of the oxygen-treated ascospores were very bulbous at the base of the spore. This may be due to a sudden germination. In both the oxygen-treated ascospores and the controls, germination occurred in less than 24 hours. Spores frozen in a dry state for a period of two months and non-frozen spores were used in the oxygen experiment. In cultures where non-frozen ascospores were used, the percentage of germination was higher and the spores were more swollen than in cultures where frozen spores were used. Dry frozen and non-frozen ascospores of *D. vernicosa* and *Daldinia X* germinated slightly better in the controls than in cultures with an excess of oxygen supply, but the ascospores of the latter were lighter brown and the germ-tubes were more granular and more swollen than were those in the controls. Therefore it seems that oxygen in excess does not increase the percentage of germination in *D. vernicosa* but it does slightly in *D. concentrica*. While an excess of oxygen causes only a slight change in color to the ascospores of *D. concentrica* and less swelling than in the controls, considerable swelling is induced and a change from a dark brown to a light brown color is observed in oxygen cultures of *D. vernicosa* ascospores. In both species the growth of mycelium is accelerated by the excess supply of oxygen. Ascospores of *Daldinia X* were also treated with an excess supply of oxygen, but they were apparently indifferent for no better germination was secured than in the controls.

EFFECT OF ENZYMES

Even though the species of *Daldinia* are not dung-inhabiting or obviously dependent upon some enzyme as a stimulus to ascospore germination, enzymatic activity might be a factor in germination, for the presence of slugs in freshly formed ascocarps of *D. concentrica* and *Daldinia X* has been frequently noted. Since in the writer's experience *D. vernicosa* has been found only once infested with slugs, it is suggested that the rarity of its occurrence may in some way be correlated with a lack of external enzyme activity.

Boudier ('69) believed that the spores of species of *Ascobulus* growing on dung germinated only after passing through the

digestive canals of animals and that the spores of species growing on the earth germinated at ordinary temperatures without special treatment. Janczewski ('71) proved that the spores of *Ascobulus furfuraceus* germinated readily after having been recovered from the faeces of an animal. The epispore was removed during the digestive process, indicating that enzymatic activity was essential to germination. There are others[1] who have successfully germinated spores in dung decoctions or have by other artificial means supplied the necessary enzyme for the dissolving of the exospore. A most interesting bit of work by Fraser ('07) on spore germination was the imitation of normal digestion which occurred when spores were placed in saliva, artificial gastric juices, pancreatic juice, and dung decoction. She found that the alkalinity supplied by the dung decoction was one of the two most important factors in germination, the other factor being heat.

One of the early investigators of spore germination was Hoffman ('60), who employed a solution of HCl and pepsin as a stimulus to germination of basidiospores, but he found that poor germination occurred. Ferguson ('02) used pepsin, distilled water, and HCl in different concentrations to stimulate spores of certain Agarics to germinate, but germination was so erratic that no conclusions could be drawn. She did find, however, that when the spores of *Agaricus campestris* came in contact with the actively growing mycelium perfect germination was secured in 144 hours, due to the secretion of an enzyme from the mycelium. Jahn ('05) has said that in the process of spore germination of the Myxomycetes the cell wall ruptures by pressure from within, but an enzyme "glycogenase" also plays a part in the process by changing the glycogen in the spore to maltose, and, since the osmosis-producing material in the newly arising protoplasm is a sugar, promotes germination. As has been asserted by Constantineau ('06), the germination process of myxomycetous spores is independent of osmotic pressure, and from a comparison of germination in isotonic solutions germination is dependent upon the ability of the spores to utilize the sugars. A more recent worker, Skupienski ('22), maintains that germination of myxomycetous spores is a purely osmotic phenomenon and not due to any enzyme activity. The most illuminating

[1] Van Tieghem ('76), Ternetz ('00), Massee and Salmon ('01, '02).

work on this subject has been done recently by Gilbert ('28), who studied 56 species and varieties of myxomycetous spores germinating in distilled water. He observed two distinct types of germination: 1, by internal pressure as exemplified by the suborder Calcarineae; and 2, probably by enzyme action, as exemplified by the order *Lamprosporales*.

The writer employed aqueous pepsin solutions of 10, 5, 2, 1, 0.5, and 0.1 per cent, in the hope of stimulating ascospore germination. The ascospores of *D. concentrica*, *D. vernicosa*, and *Daldinia X* were placed in these different solutions, but germination did not occur in any of them and the spores did not even swell except in the 5 per cent solution. The results were no more favorable when the ascospores were subjected to actively growing mycelium. The results of both of these experiments were in contrast to those of Miss Ferguson, since the spores of none of the species were influenced to greater germination. Unfortunately, the writer could pursue no further this phase of the work on enzyme activity, but it is hoped that at a later date a more complete account may be given.

EFFECT OF ULTRA-VIOLET RADIATION

While the writer was raying cultures of mycelium, it was suggested that ultra-violet rays might stimulate germination. Stevens ('28) has observed the lethal effect of ultra-violet radiation on conidia of *Glomerella cingulata* (Stoneman) Spalding and von Schrenk strain G. 10. He used full radiation from a Cooper-Hewitt quartz mercury arc operated at 4.5 amperes and 66 volts, with the petri dishes 21 cm. from the light. With the conidia on the surface of cornmeal agar, exposure for 5 seconds caused poor germination, 10 seconds, poorer germination, and after 15 seconds or more there was none. When the conidia were protected by 1.5 mm. of cornmeal agar, under exposure 5, 10, and 15 seconds poor germination followed; after exposure of 20, 25, and 30 seconds germination was only fair; 35, 40, and 45 seconds gave poor germination; 60, 70, 80, and 90 seconds gave very poor germination. The lethal effect therefore lies at slightly more than 90 seconds.

In the writer's experiments the lamp used for raying was an air-cooled Uviarc quartz lamp from Burdick Cabinet Co. The

only screen used was Vita glass from Hires Turner Glass Co. When this screen was used the rays allowed to pass were between 578 and 289 μμ (5780 A.U.—2894 A.U.).

The van Tieghem cells were prepared in the usual manner for germination experiments, and Leonian agar was used as a medium on which the ascospores, directly from the perithecia, were placed. For raying, the cover glasses with the medium and spores were carefully turned right side up and the slides were placed under the Vita glass box. After exposure, water was added to the cells, to which the cover glasses were then cemented by means of vaseline. The cultures were put in the dark and subsequently examined for germination at the end of 24 and 48 hours. All cultures were rayed for 25 seconds at 70± volts, and 42 cm. from the lamp. The results are given in table I.

TABLE I

GERMINATION OF ASCOSPORES SHOWING THE EFFECTS OF FREEZING AND ULTRA-VIOLET RADIATION. FOR EACH SPECIES AND EACH TREATMENT, 800 SPORES WERE COUNTED. LEONIAN AGAR WAS USED

Fungus	Treatment	Percentage of germination					
		Rayed			Not rayed		
		Experiments			Experiments		
		1	2	Average	1	2	Average
D. concentrica (D. Mo.)	Frozen*	95.0	83.0	89.0	87.0	69.0	78.0
	Not frozen	99.0	88.0	93.5	93.0	70.0	81.5
D. vernicosa (D. Mo.)	Frozen	2.0	2.0	2.0	4.0	4.0	4.0
	Not frozen	0.0	3.0	1.5	0.0	2.0	1.0
Daldinia X (V. P. Mo.)	Frozen	0.0	0.0	0.0	0.0	1.0	0.5
	Not frozen	0.0	0.0	0.0	1.0	0.0	0.5

* Dry ascospores frozen 2.5 months at −5° C.

This experiment with three-months-old material was divided into two phases: one in which the ascospores had been previously frozen, the other in which the material was not treated before raying. Controls were used in both cases. The percentage of germination of frozen spores of *D. concentrica* was lower in the controls (78 per cent) than in those not frozen (81.5 per cent).

While the relative germination remained proportionately the same, there was a slight increase in the percentage of germination in both groups after raying, namely 89 per cent for the frozen, and 93.5 per cent for the untreated material. *D. vernicosa*, on the other hand, reacted in quite a different manner. Freezing of the spores favored germination in the rayed and non-rayed material in this case, but the ultra-violet light inhibited spore germination. It should be noted that when the spores were not frozen and were rayed, there was a significant improvement in germination. Why this was the case is difficult to determine, although it might be that raying has much the same effect as freezing and might stimulate germination, while the ascospores which have been frozen as a result of their treatment have been made more susceptible to the action of the rays. This view seems to be supported by the fact that the rayed ascospores that did not germinate were more swollen than those not rayed. While the preceding hypothesis may hold true, there is also the possibility that the difference of .5 per cent may be laid to experimental error or irregularity in germination, yet in view of the large number (9600) of spores counted such could hardly be the case. The spores of *Daldinia X*, while germinating very poorly, were apparently little affected by freezing, although raying resulted in no germination at all.

From the above it is evident that these three species have distinct physiological reactions, and that raying, while beneficial to the ascospores of *D. concentrica*, is harmful to those of the remaining two species. Also it is clear that germination of the ascospores of *D. concentrica* is impeded by freezing, while the spores of *D. vernicosa* are favored. In general the rayed ascospores produced heavier and more frequently branched germ-tubes than did the controls, in which event it would seem that the ultra-violet rays, in the dosage used, act as a stimulus to growth.

When dry freezing of ascospores of *D. vernicosa* was observed to stimulate germination, it was suggested that even longer periods of freezing might be more effective. Hence after three months at – 5° C., cultures of *D. concentrica* and *D. vernicosa* and *Daldinia X* were made, using Pfeffer agar as the medium. Observations made after 48 hours still further confirmed the

previous evidence that freezing has a deleterious effect on the ascospores of *D. concentrica*. There was from 5 to 10 per cent more germination in the controls than in the cultures in which the frozen spores were used. The ascospores swelled considerably, as is characteristic, but the unfrozen ascospores were swollen more than the frozen ones. The germ-tubes of the unfrozen ascospores were long and unbranched, while those of the frozen ascospores were short with one to three short branches. The mycelium was heavily granular, indicating slower growth. In *D. vernicosa* 10 to 15 per cent more germination of the frozen ascospores was secured than of the controls. This is the most illuminating evidence that freezing is a stimulus to germination in this species, for in no previously tried stimuli has such a high percentage of germination been obtained. The character of the spores and germ-tubes in the culture where frozen spores were used was not significantly different from that in the controls. A third physiological reaction as represented by *Daldinia X* differs from the other two specific reactions by only a slight increase (1 to 1.5 per cent) in germination of the frozen spores over those of the controls. It would seem that this species is rather indifferent to freezing as far as the character of the ascospores and germ-tubes is concerned, for in both cases they are practically the same.

Why the ascospores of the three species should respond to freezing in so diverse a fashion is difficult to explain except that the physiological constitution of each is quite distinct. Freezing of the ascospores of the two species favorably stimulated might cause chemical changes in the ascospores, and as a result it is quite possible that the condition of the protoplasm is altered or some constituent not previously capable of being utilized may be made available by this treatment. Another possibility is that a low temperature might favor the action of some enzyme that renders reserve material available for the metabolism of the spore, preceding actual germination. Freezing may also reduce the water content of the ascospores so that the greater osmotic pressure inside may alter the permeability of the ascospore wall.

EFFECTS OF TEMPERATURE

It is evident from the preceding results that ascospores more than a year old will germinate only poorly even under a wide variety of conditions or will not germinate at all, while ascospores from one to four months old will germinate rather readily, the percentage of germination depending on the species and on the environmental factors. It is due to this fact that old material was not used in these further experiments intended to determine the factors favoring germination.

EFFECTS OF HIGH TEMPERATURES

One of the most important factors in ascospore germination of *Daldinia* is temperature. That temperature is a factor of consequence in the germination of other fungous spores has been shown by Haberlandt ('78), Müller-Thurgau ('85), Eriksson ('95), and others, who found that the subjection of the ascospores to cold for longer and shorter periods improved germination and favorably affected other life processes. That a high temperature may take the place of a food or may be the only necessary stimulus to germination is claimed by Ferguson ('02) in the case of *Hypholoma appendiculatum*. When she subjected the spores to 28° C.±, and bean decoction and distilled water were used as media, 90 and 75 per cent respectively of the spores germinated, while at 16° C. ± no germination was secured. That high temperatures for short periods of time are effective as a stimulus to germination of ascospores of *Daldinia* will be seen shortly.

When temperatures from 70 to 100° C. were employed, a double boiler containing water in both parts of the vessel was used. From a ring stand at the side of the vessel a thermometer and a small wire basket were suspended in the water of the upper part of the boiler. Under the double boiler a carefully regulated flame was maintained. The heating operations were carried on in a hood in order to avoid drafts which would prevent the maintenance of a constant temperature. The small wire basket was divided into two portions by cross wires, and small cork-plugged vials containing bits of stromatic material and perithecia were put into the basket which was lowered into the water half way up the sides of the vials. For each exposure different bits of

material from the same collection were used. Ascospores were then taken from the treated perithecia by means of a platinum needle and the usual hanging-drop culture was employed with Pfeffer agar as the medium. Ascospores of *D. concentrica* and *D. vernicosa* five months old were used in these experiments, the results of which, shown in tables II and III, represent a count of 25,400 ascospores of each species.

TABLE II

D. CONCENTRICA (D. MO.)

PERCENTAGE OF GERMINATION OF ASCOSPORES SHOWING THE EFFECT OF SHORT EXPOSURES TO HIGH TEMPERATURES. EACH EXPOSURE AND PERIOD REPRESENT A COUNT OF 600 SPORES. PFEFFER AGAR WAS USED

Temp. °C.	After 24 hrs.				After 48 hrs.			
	Experiments							
	1	2	3	Av.	1	2	3	Av.
	Exposure of 15 minutes							
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95	0.0	0.0	0.0	0.0	0.0	3.0	0.0	1.0
90	0.0	26.0	0.0	8.6	1.0	30.0	0.0	10.3
85	18.0	38.0	0.0	18.6	50.0	52.0	0.0	34.0
80	1.5	45.0	4.0	16.8	4.0	49.0	7.0	20.0
75	52.0	0.0	7.0	19.6	57.0	18.0	15.0	30.0
70	30.0	30.0	34.0	31.3	75.0	65.0	40.0	60.0
	Exposure of 10 minutes							
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95	2.0	3.0	0.0	1.6	17.0	16.0	0.0	11.0
90	2.0	1.0	0.0	1.0	13.0	5.0	0.0	6.0
85	1.5	25.0	0.0	8.8	25.0	33.0	0.0	19.3
80	50.0	49.0	5.0	36.6	56.0	54.0	10.0	40.0
75	0.0	0.0	0.0	0.0	0.0	19.0	0.0	6.3
70	20.0	50.0	17.0	29.0	22.0	54.0	22.0	36.0
	Exposure of 5 minutes							
100	0.0	0.0	0.0	0.0	4.0	0.0	0.0	1.3
95	0.0	0.0	0.0	0.0	10.0	13.0	0.0	7.6
90	50.0	33.0	0.0	27.6	56.0	40.0	0.0	35.3
85	55.0	43.0	32.6	32.6	60.0	50.0	0.0	36.6
80	56.0	53.0	5.0	38.0	60.0	57.0	11.0	42.6
75	50.0	20.0	0.0	23.3	60.0	26.0	15.0	33.6
70	25.0	24.0	23.0	24.0	28.0	30.0	30.0	29.3

Daldinia concentrica.—The highest temperature of 100° C. for 15 and 10 minutes is obviously harmful to the ascospores of *D. concentrica*, for when treated at this temperature and exposures previous to inoculation, no germination occurred even after

48 hours. Temperatures of 100° C. for 5 minutes and 95° C. for 15 minutes also have a deleterious effect, but the ascospores are not killed, for after 48 hours a very small percentage (1.3 and 1.0 per cent) of them germinated. It is interesting to note that the temperature at which the highest percentage of germination is secured after 24 hours is not the same as that at which the highest percentage of germination is secured after 48 hours. This may be seen at 80° C. for 5 minutes, at which temperature and exposure 38 per cent germination is obtained after 24 hours, while after 48 hours the maximum germination (60 per cent) occurred at 70° C. for 15 minutes. In the range 100° C. for 15 minutes—90° C. for 15 minutes, to which the ascospores were treated previous to inoculation, the swelling was only slight. Thus it appears that too high a temperature will prevent the characteristic swelling of the ascospores which attain an almost square shape, on a medium rich in carbohydrates. This same shape is attained, however, through the range of temperatures and exposures from 85° C. for 15 minutes—70° C. for 5 minutes. Although there are slight variations in the length and width of germ-tubes in the various ranges of temperatures and exposures, the length and width are correlated rather definitely. From 85° C. for 5 minutes through 75° C. for 15 minutes the average measurements are 70–100 × 1.5 μ, while at all of the temperatures and exposures above 85° C. for 5 minutes the tubes are only 25–33 × 3.3 μ. Here the mycelium shows the effects of the higher temperatures by its slow growth and densely granular content. After 24 hours the germ-tubes are not branched; 24 hours later the branching is irregular. A single germ-tube throughout the series is more common than two or an equal number of each, with the exceptions within the range of 80° C. for 10 minutes—75° C. for 5 minutes, where there are equal numbers of ascospores with one and two tubes.

Daldinia vernicosa.—The reaction of the ascospores of *D. vernicosa* to the same treatment received by those of *D. concentrica* is somewhat different. The results of this experiment are given in table III. Only 2 per cent of the ascospores germinated after 48 hours when exposed to a temperature of 100° C. for 10 minutes previous to inoculation. Nevertheless even after such an exposure the percentage of germinated ascospores is greater than that

shown in the controls. The maximum germinations, however, are obtained after the ascospores have been exposed to 75° C. for 10 minutes, when 26.6 per cent produced germ-tubes at the end of 24 hours, and 46.0 per cent after 48 hours when the ascospores were previously subjected to 70° C. for 10 minutes. Thus it becomes quite evident that heat treatment of the ascospores of this species is more stimulating than any previously tried stimuli,

TABLE III

D. VERNICOSA (D. MO.)

PERCENTAGE OF GERMINATION OF ASCOSPORES SHOWING THE EFFECT OF SHORT EXPOSURES TO HIGH TEMPERATURES. EACH EXPOSURE AND PERIOD REPRESENTS A COUNT OF 600 SPORES. PFEFFER AGAR WAS USED

Temp. °C.	After 24 hrs.				After 48 hrs.			
	Experiments							
	1	2	3	Av.	1	2	3	Av.
	Exposure of 15 minutes							
100	0.0	0.0	0.0	0.0	0.0	1.0	5.0	2.0
95	0.0	8.0	9.0	5.6	13.0	12.0	50.0	25.0
90	0.0	18.0	16.0	11.3	30.0	36.0	25.0	30.3
85	0.0	20.0	20.0	13.3	30.0	32.0	32.0	31.3
80	0.0	55.0	2.0	19.0	0.0	60.0	38.0	32.0
75	6.0	40.0	0.0	15.3	30.0	50.0	43.0	41.0
70	0.0	24.0	7.0	14.0	50.0	51.0	15.0	38.6
	Exposure of 10 minutes							
100	0.0	1.0	1.0	0.6	14.0	6.0	14.0	11.3
95	0.0	9.0	11.0	6.6	46.0	16.0	30.0	30.6
90	4.0	8.0	9.0	7.0	38.0	34.0	26.0	32.6
85	0.0	23.0	22.0	15.0	50.0	39.0	38.0	42.3
80	0.0	54.0	20.0	24.6	36.0	58.0	36.0	43.3*
75	0.0	50.0	30.0	26.6	20.0	58.0	45.0	41.0
70	0.0	50.0	20.0	23.3	41.0	59.0	40.0	46.0
	Exposure of 5 minutes							
100	0.0	2.0	5.0	2.3	9.0	7.0	25.0	13.6
95	0.0	12.0	14.0	8.6	41.0	20.0	24.0	28.3
90	5.0	25.0	17.0	15.6	40.0	44.0	28.0	37.3
85	1.0	13.0	12.0	8.6	40.0	35.0	40.0	38.3
80	2.0	27.0	50.0	26.3	40.0	38.0	60.0	46.0
75	1.0	30.0	5.0	12.0	50.0	44.0	40.0	44.6
70	0.0	20.0	22.0	14.0	40.0	38.0	43.0	40.3

* Conidia after two weeks.

including freezing of the dry ascospores for three months, which treatment only caused 10–15 per cent to germinate. The ascospores never under any circumstances attain the square shape

after swelling, such as is characteristic of the ascospores of *D. concentrica* on a medium rich in carbohydrates, but swelling of the ascospores previously heated at 95° C. for 10 minutes, 90° C. for 15 minutes, 85° C. for 10 minutes, and 80° C. for 5 minutes, is more pronounced than under any previous conditions. Here the ascospores are almost spherical. At higher temperatures, from 100° C. for 15 minutes through 95° C. for 15 minutes, the ascospores are only slightly swollen and at other temperatures and exposures the swelling is considerably more than in the controls where the ascospores were not heated. The germ-tubes also respond in a rather definite manner to the different intensities of heat to which the ascospores are treated previous to inoculation. The germ-tubes which arise from the ascospores exposed to 100° C. for 15 minutes through the intervening temperatures and exposures to 90° C. for 5 minutes are very short and wide, 8–10 × 4.4 μ, while the somewhat longer exposures of the ascospores, 90° C. for 10 minutes through 85° C. for 5 minutes, produced more extensive and narrower germ-tubes, 20–46 × 3.3 μ. The germ-tubes of the ascospores treated at 80° C. for 15 minutes through the intervening temperatures and exposures to 70° C. for 5 minutes grew more rapidly than any of the others; here they were 60–72 × 2.2 μ. In contrast to *D. concentrica* the germ-tubes do branch, one to four times after 24 hours. However, no generalization can be made due to the fact that the branching is too erratic. Branching soon after the germ-tube has grown out from the ascospore is much more common in *D. vernicosa* than in *D. concentrica* under any condition. With one exception, a single germ-tube throughout this series is also more common than two or an even number of one and two. An even number of one and two germ-tubes was produced by the ascospores exposed to 80° C. for 5, 10, and 15 minutes. In comparing the ranges of the two species at which an equal number of one and two germ-tubes occurs, it is seen that they are close together, overlapping at 80° C. for 5 minutes, with *D. concentrica* extending through 75° C. for 5 minutes. Thus it appears that at the temperatures and exposures at which the most rapid growth occurs, in *D. concentrica* at least, an equal number of one and two tubes is found. This does not seem to hold true for *D. vernicosa*, since

the most rapid growth is noted in the germ-tubes from the ascospores which were exposed to 70° C. for 10 and 15 minutes.

A comparison of germination of the two species at the different temperatures and exposures is given graphically in fig. 1. The ascospores of *D. concentrica* do not germinate at the highest temperatures here employed and the high percentages are more uneven in their occurrence than in *D. vernicosa*. For *D. concentrica* 5

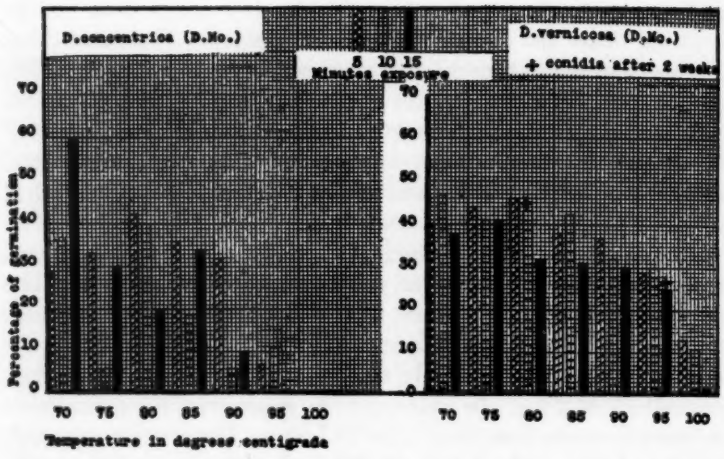

Fig. 1. Ascospore germination showing the effect of short exposures to high temperatures previous to inoculation.

minutes seems to be the optimum exposure, since a more even increase in percentage of germination is indicated, but the maximum percentage is secured at 15 minutes exposure at 70° C. The longest exposure, 15 minutes, appears to be the optimum for *D. vernicosa*, but there are two maxima, one at 80° C. for 5 minutes and the other at 70° C. for 10 minutes; at both exposures 46.6 per cent germination occurred. It is noted in both species that the percentage of germination increases as the temperature and exposure decrease, but even the maximum of 60 per cent germination in *D. concentrica* is not as high as in the controls where 85–90 per cent germination was secured. In both of the species the growth of the germ-tubes is retarded by the exposure to

higher temperatures. This is evident from the fact that the germ-tubes are short and wide with densely granular cell content.

After two weeks the cultures were again examined, this time for conidia. They were found fully developed only in those cultures of ascospores that had been exposed to a temperature of 95° C. for 15 minutes; immature conidia were also found in *D. vernicosa* in those cultures the ascospores of which had been previously treated at 80° C. for 10 minutes, and not at all in *D. concentrica*.

To summarize, it is apparent that short exposures to high temperatures previous to inoculation favorably affect the germination of ascospores of *D. vernicosa*. While the percentage of germination increases as the temperature is lowered from 100° to 70° C. there is a much greater percentage of germination even at 100° C. than in the controls. That an increase in germination occurs when the ascospores are heated is significant when correlated with the fact that *D. vernicosa* usually grows on charred wood. One of the reasons for its rare occurrence is no doubt the fact that its ascospores rarely meet with favorable conditions for germination. Temperature, either high or low, is evidently a very important factor in germination. Whether the inability of the ascospores to germinate is due to the slightly heavier spore wall than that of *D. concentrica* or to a different physiological constitution is difficult to determine. It is a chemical fact that in general an increase in temperature increases chemical activity, and it is also a physical fact that higher temperatures stimulate physical activities which are manifest in swelling. Heat in all of the intensities used probably accelerates chemical activities in the ascospores of *D. vernicosa*, resulting in a higher percentage of germination. The effects of accelerated physical activities are most pronounced in the ascospores treated from 80 to 95° C., at which temperatures the ascospores swelled to a spherical shape. Hence in the case of both species, it seems that short exposures at high temperatures (90° C.) have approximately the same effect as longer exposures at lower temperatures (70–75°C.). At any rate the results of this experiment are illustrative of the fact that there is a decided distinction in the requirements for germination of the two species.

EFFECT OF RELATIVELY HIGH TEMPERATURE IN RELATION TO GROWTH ON VARIOUS MEDIA

The effect of exposing ascospores to high temperatures for short periods of time previous to inoculation has been demonstrated. The object of this experiment is to determine the effect of exposure to relatively high temperatures after inoculation. As a source of nutrition, Leonian, Pfeffer, oatmeal, prune, and *Platanus* decoction agars were used. As a source of heat constant-temperature ovens at 38° C. and at 25° C. were used. *D. concentrica*, five months old, was the only species tested in this experiment. The results are recorded in table IV.

TABLE IV

D. CONCENTRICA (D. MO.)

GERMINATION OF ASCOSPORES SHOWING THE EFFECT OF DIFFERENT MEDIA AND A CHANGE IN TEMPERATURE. THE RESULTS REPRESENT A COUNT OF 800 SPORES ON EACH MEDIUM

Agar medium	Percentage of germination					
	Period of growth					
	24 hrs. at 38° C.			48 hrs at 25° C.		
	Experiments					
	1	2	Average	1	2	Average
Pfeffer	0.0	0.0	0.0	81.0	85.0	83.0
Leonian	11.0	13.0	12.0	29.0	32.0	30.5
Oatmeal	16.0	16.0	16.0	Very good germination; impossible to count percentage because of dense mycelial growth.		
Prune decoction	0.0	0.0	0.0	38.0	32.0	35.0
Platanus decoction	0.0	0.0	0.0	57.0	55.0	56.0

On all of the media after 24 hours at 38° C. it became apparent that the temperature was too high for optimum germination. Those ascospores which had not germinated were apparently plasmolyzed, as indicated by the concavities and large oil globules which had appeared. After counts were made the cultures were put into an oven at 25° C. and counts were again made after 48 hours.

On Leonian agar after 24 hours at 38° C., only 12 per cent of the ascospores had germinated, but all of them were swollen. That the temperature was too high was evident by the low percentage of germination and the germ-tubes which averaged only 18 μ in length and 4.4 μ in diam., and were very granular, and not

branched. After 48 hours at 25° C. there was only 30.5 per cent germination; the other ascospores were plasmolyzed. The germinated ascospores were considerably swollen and the mycelium was short and thick. The ascospores on Pfeffer agar were seriously affected by 38° C., for none of them germinated after 24 hours and they were either only slightly swollen or else plasmolyzed. After the cultures had been put into 25° C., the ascospores recovered from their state of partial plasmolysis, and 83 per cent of them germinated on Pfeffer solution agar. The germinated ascospores swelled to a square shape and the growth of the mycelium, 3.4 μ diam., was characteristically poor. After 24 hours on oatmeal agar the ascospores were considerably swollen, and although 16 per cent germination was secured the mycelium was abundant and 4.4 μ in diam. After transferring to 25° C. the mycelium was so abundant that a count for germination was impossible, although quite obviously the percentage was greater than 50 per cent. Seemingly, because of the high temperature and the high sugar content of the prune-decoction agar, all of the ascospores were plasmolyzed after 24 hours at 38° C., and no germination occurred. After 48 hours at 25° C. 35.5 per cent of the ascospores germinated, were considerably swollen, and the mycelium was heavily granular and much enlarged at the ascospore. The high sugar content of the medium is conducive to short thick and granular mycelium, characteristic of slow growth, the largest attaining an average of 7.2 μ in diameter, while on other media (oatmeal) only 4.4 μ. The ascospores on *Platanus* decoction agar at 38° C. behaved very much the same as those on prune-decoction agar, for after 24 hours none of the ascospores had germinated and were either plasmolyzed or only slightly swollen. On exposure for 48 hours at 25° C., however, the ascospores recovered from their state of partial plasmolysis and 56 per cent germination was obtained. The germinated ascospores had not swollen any further, and the growth of the mycelium was poor, only 3.3 μ diam., and not granular.

From the results of these experiments it is evident that the ascospores will not germinate at as high a temperature as 38° C. after inoculation. This appears to be due to the fact that the ascospores, although able to absorb moisture from the medium,

cannot grow, since heat inactivates the necessary enzymes. The absorption of certain constituents from the medium as a result of the high temperature may also cause in the ascospores a chemical change which is toxic. That the ascospores may recover from a partial state of plasmolysis is shown by changing the cultures to an oven where the atmosphere is 13° C. cooler, since after 48 hours at a temperature of 25° C., the ascospores germinated. Pfeffer agar gives the highest percentage of germination (83 per cent) and *Platanus* decoction agar gives the next best with 65 per cent. While Pfeffer agar is the best medium for ascospore germination, it is the poorest for mycelial growth. Oatmeal agar has proved to be the best medium for mycelial growth and is also probably one of the best media for germination of *D. concentrica* ascospores, although they are so completely enmeshed in mycelium after 72 hours that a spore count is almost impossible.

EFFECT OF DIFFERENT MEDIA

That there is an essential difference between a medium favorable for fungous spore germination and one favorable for growth, Duggar ('01) has noted. He asserts that in general a perfect food is the best stimulus for spore germination of saprophytic forms. From the immediately preceding results it is also noted that there is a difference between a germination medium (Pfeffer agar) and a growth medium (oatmeal agar), but in the case of *D. vernicosa* the stimulus seems to be more important than the food. In studying the germination of a large number of myxomycetous spores, Constantineau ('06) came to the conclusion that carbohydrates alone were insufficient to give good results, although sucrose was a better sugar for germination than glucose. Experiments showed that decoctions of substrata on which the Myxomycetes naturally occurred were favorable for germination. Decoctions of *Platanus* wood, a substratum on which *Daldinia* is found frequently in Missouri, proved to be a favorable medium for germination. The requirements which Maneval ('26) found essential for germination of rust teliospores were oxygen supply, temperature, and maturity, while the substratum and light were of minor importance. This is not true of *Daldinia* ascospores, as is shown by the following tests to determine which of the agar

media used gave the maximum germination at room temperature. The character of the germ-tubes, swelling of the spores, etc. were not regarded here. Material of *D. concentrica*, *D. vernicosa*, and *Daldinia X* was used.

Daldinia concentrica ascospores germinated favorably on all of the media, but Pfeffer solution was the best (80.6 per cent), *Platanus* decoction next (54 per cent), oatmeal third (51.5 per cent), Leonian fourth (47.3 per cent), and prune decoction least favorable with 37.6 per cent germination. According to Wehmer

TABLE V

GERMINATION OF ASCOSPORES SHOWING THE EFFECT OF DIFFERENT MEDIA AT ROOM TEMPERATURE. THE RESULTS REPRESENT A COUNT OF 1200 SPORES FOR EACH SPECIES AND ON EACH MEDIUM

Agar medium	*D. concentrica* (D. Mo.)				*D. vernicosa* (D. Mo.)				*Daldinia X* (V. P. Mo.)			
	Percentage germination after 48 hrs.											
	Experiments											
	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
Pfeffer	76.0	85.0	81.0	80.6	3.4	4.0	2.5	3.3	1.5	1.0	1.1	1.2
Leonian	47.0	49.0	46.0	47.3	1.0	1.0	1.0	1.0	.7	.4	.7	.6
Oatmeal	54.0	49.0	52.0	51.5	1.1	1.4	1.1	1.2	.6	.5	.7	.6
Prune decoction	35.0	41.0	37.0	37.6	.6	.6	.3	.5	.4	.3	.2	.3
Platanus decoction	54.0	57.0	51.0	54.0	0.0	0.0	0.0	0.0	0.0	0.0	0,.0	0.0

('11), who analyzed the dried fruit of *Prunus domesticus* L., the invert sugar which contains an equal number of glucose and fructose molecules is present in from 23 to 56 per cent and sucrose in 3.9 per cent, but in the dilution of the prune decoction employed in these experiments only approximately 4 per cent sugar is present. Leonian agar, however, is somewhat richer in carbohydrates, for it contains 12.5 per cent maltose and malt extract. From the results with these two agars one would be led to believe that germination is not favored by a medium which contains carbohydrates. However, the results with maltose solutions, as will be shown subsequently, are contradictory to this supposition, for in a 10 per cent solution a high percentage of germination was secured.

The analysis of young twigs and buds of *Platanus occidentalis* have been shown by Wehmer ('11) to vary with the time of year. In analyses made in October, which coincides more nearly to the date of collections by the writer, the following percentages of constituents were given: 44.6 CaO, 7.3 K_2O, 20.0 SO_3, 4.3 P_2O_5, 1.4 Na_2O, 19.0 SiO_2, 1.19 $Fe_2O_3 + Al_2O_3$, 3.4 MgO, 1.7 N, and 10.87 ash. From a decoction of *Platanus* twigs, then, according to this analysis, there are no sugars present. This fact may account for the superiority of *Platanus* decoction over prune decoction and Leonian agars. The reasons for the superiority of Pfeffer agar over Leonian can be deduced, for the composition of these media are more definitely known. Both of these media contain KH_2PO_4 and Mg SO_4, but Pfeffer solution contains 1.00 gram of KH_2PO_4, and 1.00 gram of $MgSO_4$ per liter, while Leonian contains .625 grams of KH_2PO_4 and .625 grams of $MgSO_4$ per liter. Leonian also contains maltose and malt extract as well as peptone which is both a source of carbon (from the lactose present in the meat) and nitrogen (from the proteins), while Pfeffer agar contains no sugars except for the mere trace that may be present in the agar. However, Pfeffer agar contains two sources of nitrogen, in the form of nitrates $Ca(NO_3)_2$ in the proportion of 1.33 grams per liter, and KNO_3 in the proportion of ⅓ gram per liter. From this, it may be deduced that nitrogen must be the constituent which is most valuable in the form of a nitrate. Benecke ('95) considered potassium absolutely necessary for the germination of myxomycetous spores, and when one considers that Pfeffer agar has three different sources amounting to 2.5 grams, while Leonian agar has only one source of 1.25 grams, it seems entirely possible that the potassium as well as the nitrogen source makes Pfeffer's a better medium for germination.

To summarize briefly the results of this experiment, both *D. vernicosa* and *Daldinia X* ascospores germinated better in Pfeffer agar than in any other medium, but the percentages were very low, with only 3.3 per cent of the former and 1.2 per cent of the latter. It is interesting to note that no germination was obtained on *Platanus* decoction agar, which seems to be a perfectly good reason why *D. vernicosa* and *Daldinia X* are not found on *Platanus*. The writer has frequently found *D. concentrica* on *Platanus*

but never the other two species. Leonian and oatmeal agars seemed to be equally good for germination of *Daldinia X*, but oatmeal gave .2 per cent better germination than Leonian for *D. vernicosa*. With the exception of *Platanus* decoction agar, prune-decoction agar for both species appears to be least favorable. If one recalls that a stimulus of heat is necessary for maximum germination of *D. vernicosa*, the low percentages obtained here where no heat was used may be partially explained. As for *Daldinia X*, neither the appropriate stimulus nor medium appears to have been found.

EFFECT OF MALTOSE SOLUTIONS

When sucrose solutions alone were employed as a medium, old ascospores were used, and although considerable swelling was induced no germination occurred. It has been noted that swelling of ascospores is more pronounced on a medium rich in sugars than on one which contains little or no sugar. Since swelling to any extent in general is an indication of likeliness to germinate, a nutrient solution of maltose alone in distilled water was employed. It is known that maltose is a nutritive substance of great value and in living organisms is transferred into assimilable sugar more rapidly than is sucrose. While no particular attempt was made to determine which of the sugars was better for germination, maltose proved to be superior to sucrose. Other investigators have found that sucrose is superior to glucose for germination. Constantineau ('06) found this to be true in an extensive study made on myxomycetous spores. The ability of fungous spores to utilize sugars is so specific that it is a matter of experimentation to determine which disaccharide or which monosaccharide sugar is most available or assimilable.

Three different ranges of maltose solutions were employed in order to secure a fair representation of a most probable range of concentrations favorable for germination. The solutions were sterilized in the autoclave at 15 pounds pressure for half an hour and the usual hanging-drop method of culture adopted. Upon autoclaving the maltose molecule partially breaks down into two glucose molecules, while that of sucrose gives one of glucose and one of levulose. That the two glucose molecules may be more

available as a source of carbon to the ascospores than one of glucose and one of levulose is one factor which probably enters into consideration.

In this experiment ascospores of *D. concentrica*, *D. vernicosa*, and *Daldinia X* one year old and one month old were used, except in the case of *Daldinia X* where the only available material was one month old. The different ages were used in order to

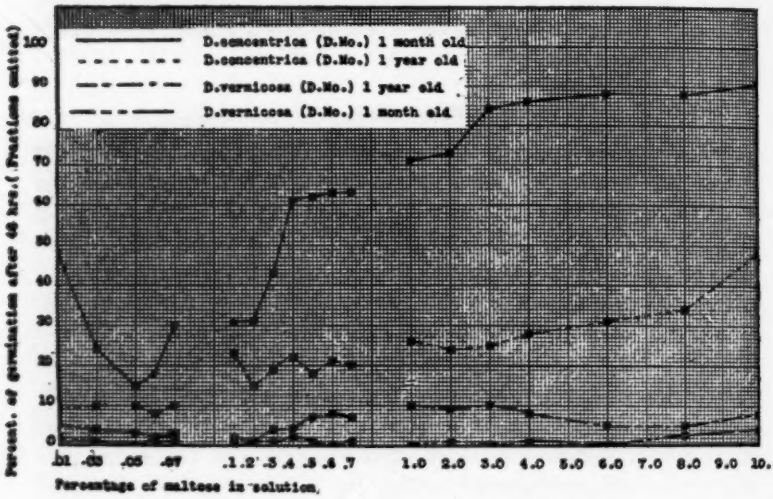
D.concentrica (D.No.) 1 month old
D.concentrica (D.No.) 1 year old
D.vernicosa (D.No.) 1 year old
D.vernicosa (D.No.) 1 month old
Percentage of maltose in solution.

Fig. 2. Germination of ascospores in maltose solutions.

determine whether or not "ripening" or maturation was a factor in germination. The one-year-old material which had been kept in the writer's herbarium had not been fumigated or treated with any disinfectant, nor had the month-old material. The results are given graphically in fig. 2 and in table VI.

Daldinia concentrica.—The percentage of germination in the year-old material from .01 to .07 per cent maltose solutions varied very little, only from 10.6 per cent secured at .03 per cent solution to 8.0 per cent secured at .06 per cent solution. Within this same range the ascospores were considerably swollen, but at higher concentrations from .1 to .7 per cent solutions the swelling was more pronounced. At these concentrations there

TABLE VI

GERMINATION OF ASCOSPORES AFTER 48 HOURS IN MALTOSE SOLUTIONS. IN THE YEAR-OLD MATERIAL, 12,000 ASCOSPORES OF EACH SPECIES WERE COUNTED, WHILE IN THE MONTH-OLD MATERIAL, 8,000 ASCOSPORES WERE COUNTED FOR EACH SPECIES

Percent maltose solution	Percentage of germination													
	D. concentrica (D. Mo.)							*D. vernicosa* (D. Mo.)						
	1 year old				1 month old			1 year old				1 month old		
	Experiments							Experiments						
	1	2	3	Av.	1	2	Av.	1	2	3	Av.	1	2	Av.
.01	10.	5.	12.	9.	46.	50.	48.	11.	3.	3.	5.6	3.	1.	2.
.03	12.	7.	13.	10.6	34.	14.	24.	2.	8.	4.	4.6	1.	0.	.5
.05	10.	10.	10.	10.	15.	16.	15.5	5.	0.	5.	3.3	0.	0.	0.
.06	12.	4.	8.	8.	4.	30.	17.	4.	2.	0.	2.	2.	1.	1.5
.07	20.	6.	5.	10.3	32.	28.	30.	0.	5.	0.	1.6	3.	3.	3.
00.1	15.	10.	46.	23.6	26.	36.	31.0	2.	0.	0.	0.6	2.	2.	2.
00.2	14.	12.	30.	14.6	20.	40.	31.	4.	0.	0.	1.3	0.	2.	1.
00.3	13.	13.	23.	19.6	43.	44.	43.5	2.	8.	4.	4.6	1.	1.	1.
00.4	13.	15.	38.	22.6	64.	58.	61.	3.	9.	0.	4.	1.	3.	2.
00.5	16.	18.	20.	18.	64.	60.	62.	2.	0.	2.	7.3	0.	3.	1.5
00.6	21.	21.	21.	21.	68.	59.	63.5	4.	20.	0.	8.	0.	1.	0.5
00.7	25.	23.	24.	24.	66.	60.	63.	0.	18.	0.	6.	0.	2.	1.
1.00	26.	26.	27.	26.3	68.	74.	71.	10.	22.	0.	10.6	0.	0.	0.
2.00	24.	26.	22.	24.	67.	80.	73.5	3.	25.	0.	9.3	0.	2.	1.
3.00	20.	30.	25.	25.	85.	84.	84.5	5.	27.	0.	10.6	1.	0.	0.5
4.00	16.	30.	40.	28.6	87.	86.	86.5	4.	20.	0.	8.	3.	0.	1.5
6.00	20.	31.	43.	31.3	88.	88.	88.	11.	3.	1.	5.	0.	0.	0.
8.00	25.	33.	44.	34.	90.	87.	88.5	13.	7.	0.	6.6	4.	3.	3.5
10.00	50.	46.	52.	49.	93.	90.	91.5	12.	12.	2.	8.6	3.	5.	4.
Sterile distilled water	16.	17.	16.	16.5	17.	19.	18.	1.	1.	1.	1.	0.	0.	0.

was also a higher percentage of germination, but the percentages were more uneven in their occurrence. At .1 per cent solution there was 23.6 per cent germination, while at .2 per cent solution there was only 14.6 per cent germination, but from .3 to .7 per cent there was an increase again to 24.0 per cent at the concentration of .7 per cent. Germination at 1 per cent solution of maltose reached 26.3 per cent, and from 4 to 10 per cent solution there was a steady increase from 28.6 to the maximum, 49 per cent, germination reached at 10 per cent solution. At these higher concentrations, from 1 to 10 per cent, the ascospores were swollen to a square shape. As to the germ-tubes, a single one was most common throughout the whole series, and most of them were 1.5 μ wide, but the length varied with the concentrations of solutions. From .01 through .4 per cent solutions, the germ-tubes were 175

to 300 × 1.5 to 1.8 μ; from .5 through 4 per cent the tubes were longer, 300 to 500 × 1.5 μ; from concentrations of 6 through 10 per cent there was a variation from 125 to 400 μ. The longest ones (400 μ) in this last series occurred in the 10 per cent solution. The branching of the germ-tubes was more consistently confined to the concentrations from .01 through .03 per cent, while from .05 through .1 per cent they were not branched, as was also true in the solutions from .2 through 10 per cent maltose concentrations.

The month-old material presented an entirely different curve which exceeded the preceding one in height by a difference of 42 per cent germination in the 10 per cent solution. The whole germination curve of the new material was less undulating, except in the lowest concentrations from .01 through .07 per cent. At the former concentration 48 per cent germination occurred, at the latter 30 per cent. The minimum germination in this series was 15.5 per cent, which occurred at .05 per cent solution. The ascospores in this series were only slightly swollen, while in the remaining concentrations the swelling was characteristically square in shape. With an increase in concentrations from .1 through .7 per cent a marked increase from 31 to 63 per cent germination was secured. The germination curve straightened out from 1 through 10 per cent, starting at 71 per cent germination and rising through 73.5, 84.5, 86.5, 88.0, 88.5 per cent, until the maximum percentage of 91.5 per cent was reached. Throughout the series, as in the old material, one germ-tube was most typical, although in the .7 per cent solution, two were more common than one. The two lowest concentrations were least favorable to growth of germ-tubes from ascospores of fresh material, for the tubes averaged 65 × 1.8 μ, and 30 × 1.5 μ. At slightly higher concentrations of solutions from .05 through .7 per cent the average tube ranged from 100 to 30 × 1.5 μ. Within the short range of solutions from 1 through 4 per cent, the germ-tubes were of uniform length and diameter, 500 × 1.5 μ. At the three highest concentrations, growth was less rapid, with only 175 μ in length but the same width. It will be noted that the diameter remains uniform throughout the series except at .01 per cent where there was a difference of .3 per cent.

Thus from a comparison of these results it is seen that the

behavior of the younger material is much more consistent than that of the older. This is true not only of the percentages of germination, but the length and diameter of germ-tubes. Branching of the germ-tubes is under any condition more or less erratic, but the highest concentrations seemed to induce slower growth and no branching, which facts appear to be correlated with the highest percentages of germination. The ascospores are probably able to assimilate the higher percentages to more advantage than the lower percentages. In both cases the higher concentrations of maltose induced more swelling than the lower concentrations. In the controls where sterile distilled water was used the fresher ascospores germinated somewhat better than the older ones. In the former there was 18 per cent germination, while in the latter, 16.5 per cent. The ascospores in both cases were only slightly swollen and the single germ-tubes were not branched. The germ-tubes of the older ascospores were 300 × 1.5 μ, while those of the younger were only 100 × 1.5 μ. Evidently the ascospores of *D. concentrica* need no period of rest. "Ripening," or maturation, for maximum germination is secured when comparatively fresh material is used. This is still further proof for the supposition that the ascospores germinate soon after discharge from the perithecia, in the late summer or early fall, and that the fungus lives over the winter period in the vegetative stage. In order to gain more evidence for this hypothesis the effect of freezing the mycelium will be investigated at a later date.

Daldinia vernicosa.—The case of *D. vernicosa* presented almost the opposite picture of *D. concentrica* as seen in fig. 2. From this, it is apparent that in order to secure the optimum germination, a "ripening" or maturation is necessary. Whereas a treatment of artificial wintering by freezing or a heat treatment gave maximum germination, a resting period of a year or so was also effective. The percentages of germination were low when the ascospores were not treated, with a minimum of .6 per cent at .1 per cent solution of maltose and a maximum of 10.6 per cent at 1 and 3 per cent solution. When treated with heat before inoculation as demonstrated previously, 46 per cent germination was secured. In the older material, as a whole, the range of maltose concentrations from 1 through 10 per cent appeared to

be more conducive to germination than the lower concentrations. The lowest concentrations from .01 through .07 per cent induced the least swelling, the second range of concentrations from .1 through 4 per cent induced more pronounced swelling, and the highest concentrations from 4 through 10 per cent stimulated the ascospores to swell to an almost spherical shape. With the exception of from .4 through .7 per cent solution, the ascospores were as light a brown after swelling as they were when treated with H_2O_2. It seems barely possible that the dark brown endospore became oxidized in this case. The maltose solutions at these concentrations may have been less active chemically and thus have prevented a change in color. The number of germ-tubes was very consistent in this group, with an equal number of one and two tubes from .01 through .07 per cent, and in all of the other concentrations one tube was most common. The size of the germ-tube varied considerably. From .01 through .5 per cent solution they were 100 to 195 × 1.8 to 3.3 μ, and although the diameter of the tubes remained constant, 3.3 μ, from .6 through 4 per cent, the length varied from 300 to 500 μ. The germ-tubes in the three remaining concentrations of maltose solution varied from 100 to 200 × 1.8 to 2.7 μ. As for the branching of the germ-tubes, the series of concentrations was divided into two parts with little variation; from .01 through .3 per cent the tubes were mostly unbranched and from .4 through 10 per cent they were branched.

The younger material of *D. vernicosa* gave considerably less germination than the older. The maximum percentage of 4.0 per cent was secured in the 10 per cent solution. From the results which are represented graphically in fig. 2, it is seen that the percentage of germination presents an almost straight line. At three concentrations, .03, .6, and 3 per cent, only .5 per cent germination was obtained, while no germination at all was secured at .05, 1, and 6 per cent. Here, as in the older material, definite ranges of concentrations gave different degrees of swelling. At the lower concentrations from .01 through .6 per cent, the ascospores were only slightly swollen, and from .7 through 6 per cent they were considerably swollen. Only at the two highest percentages were the ascospores swollen to a spherical shape.

The range at which an equal number of one and two tubes occurred was almost identical with that of the older material, but one exception occurred at .07 per cent in the younger material where two tubes were more common. The remainder had single tubes. From .01 through .07 per cent solution the germ-tube length and diameter were very consistent with the measurements 200 × 2.8 μ, but from .1 through 6 per cent solution there was a variation from 100 to 200 × 3.3 μ. At the two highest percentages of solutions the narrowest mycelium occurred, and the branches were longer than in any of the preceding concentrations. The germ-tubes averaged 100 × 1.5 μ. Branched germ-tubes were present in all of the concentrations.

In comparing the results of germination of the year-old and the month-old ascospores of *D. vernicosa*, it is evident that the older ones are benefited by their period of "rest." Whereas the percentage of germination in both cases is increased in a 10 per cent solution of maltose, the maximum germination of the older material was not secured at 10 per cent but at 3 per cent and 1 per cent. While the concentrations in which the different degrees of swelling of the ascospores occurred were somewhat different, the degree of swelling was practically the same. The ranges of solutions in which equal numbers of one and two tubes and single tubes occurred were practically the same. In general, the germ-tubes of the older material grew more rapidly than those of the younger, but there was less variation in the length and diameter of the latter than the former. In the younger material where the tubes were not as long as in the older, branching occurred throughout the whole series. In the controls where sterile distilled water was used, none of the new material and only 1 per cent of the older material germinated. In the former the ascospores did not swell at all, but in the latter the swelling was slight and the unbranched germ-tubes averaged 150 × 3.3 μ.

Daldinia X.—Daldinia X, the ascospores of which were about two months old, still further showed a physiological difference by not germinating at all in the maltose solutions. The cultures were kept under observation for six days and, although slight swelling occurred in the lower percentages and more swelling was noted at the four highest percentages, no germination was observed.

Thus a comparison of the results of these experiments still further confirms the differences in the three species and their differences in physiological reactions at different ages.

EFFECT OF DIFFERENT HYDROGEN-ION CONCENTRATIONS ON ASCOSPORE GERMINATION

It has already been seen that the source of nutrition is a factor of great consequence in the germination of ascospores. Maltose, it was found, at a concentration of from 1 to 10 per cent favored germination. Also it has been shown in this paper that Pfeffer solution agar is far more conducive to germination than any of the other media tried. The reasons for this are suggested by Coons ('16), who found that carbohydrates in excess make a medium acid while proteins in excess make it alkaline. With such results it is obvious that hydrogen-ion concentration should be a factor of consequence in ascospore germination.

The toxicity of OH and H ions in this relation has been determined by Clark ('99) for certain mould fungi. He found that certain acids retarded germination and also the early mycelial development. In addition, he demonstrated that the hydroxyl ions were more toxic than the hydrogen ions.

More extensive work concerning the influence of hydrogen-ion concentration on germination of fungous spores was done by Webb ('19, '21), who found that by increasing the concentration in a cultural solution consisting of NaOH, H_3PO_4, and M/5 mannite, the germination of spores of *Fusarium* sp., *Lenzites sepiaria*, *Aspergillus niger*, and other fungi was favorably increased. In further investigations he found that by increasing the acidity of mannite, peptone, and Czapek's solution, sugar beet decoction, "H_2O, H_3PO_4, and NaOH," and "H_2O, HCl, or KOH" from neutrality to approximately pH 3.0–4.0 germination of *Lenzites sepiaria* was stimulated. Beet decoction gave the best range and percentage of germination, while "H_2O, H_3PO_4, and NaOH" gave the poorest. In equal concentrations the OH ions were more toxic than the H ions. The direction and magnitude of the change in the reaction of the medium due to spore germination depend upon the fungus, medium, and initial reaction of the solution. He further says that spore germination is

a process which is strikingly supported by conditions of active acidity; relatively low percentages of germination in most cases are obtained under conditions of active alkalinity. Also the fact is emphasized that the influence of the hydrogen-ion concentration on the germination of spores is dependent upon the nature of nutritive material present in the medium.

Therefore, because of the importance attached to hydrogen-ion concentration it appeared necessary for the sake of completeness to ascertain the influence of hydrogen-ion concentration on the ascospores of *Daldinia*. For this purpose, since material of

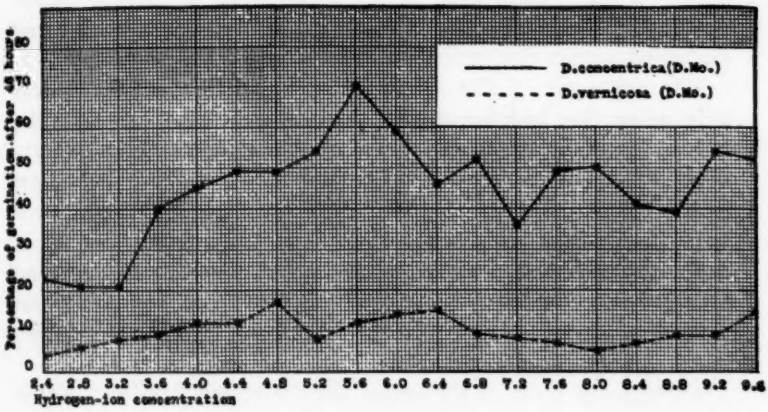

Fig. 3. The effect of different hydrogen-ion concentrations on ascospore germination.

Daldinia X was not available at the time, only the ascospores of *D. concentrica* and *D. vernicosa* were employed; these were five months old. The medium used was Pfeffer's solution made solid by the addition of 3 per cent agar, and adjusted either by citric acid or NaOH. A range of hydrogen-ion concentrations from 2.4 to 9.6 with intervals of .4 was thus obtained. The results given in table VII for *D. concentrica* and in table VIII for *D. vernicosa* are averages of three different experiments representing a count of 22,800 ascospores of each species. Figure 3 represents the percentage of germination of ascospores, but after 48 hours.

Daldinia concentrica.—After 24 hours the percentage of germination rose gradually from 12 per cent at pH 2.4 through 13, 14.3, 19, 22.3, 26.3, 46.3, and 54 per cent, the third highest percentage until the maximum was reached at pH 5.6 where 70 per cent germination was secured. The second highest percentage, 59 per cent, was reached at pH 6.0. At the end of 48 hours these maxima remained the same, no additional ascospores being found to have germinated. At the extreme alkaline end of the series (pH 9.6) 35.0 per cent germination was secured. As is shown by fig. 3, which represents the percentage of germination after 48 hours, there was an increase over that obtained after 24 hours. Nevertheless the range in which the highest percentages were secured remained within the narrow limits from pH 5.2 to 6.0.

The character of the ascospores and germ-tubes was noted only after the 24-hour period. At this time throughout the whole series of hydrogen-ion concentrations, the ascospores swelled to an almost square shape with the exception of a narrow range on either side of neutral. This range included the hydrogen-ion concentrations from pH 5.6 through 7.6, in which all of the ascospores were swollen but only the germinated ones had attained the characteristic square shape. Thus it appears that extreme acidity or alkalinity will induce swelling of the ascospores, whether they germinate or not.

Throughout most of the series the ascospores produced either one or two germ-tubes, the number of which were about equally divided. At pH 6.0, 6.4, and 8.8, however, the ascospores most commonly produced two germ-tubes, a fact which is indicative of a fair degree of correlation with the optimum of germination. At the same time the growth of germ-tubes was most rapid at pH 7.6 at which point the average size was 325 × 4.4 μ. On the other hand, growth was least rapid at pH 4.0, only 39 × 3.3 μ. At the remaining concentrations the germ-tubes were mostly 100 × 3.3–4.4 μ. Between the hydrogen-ion concentrations of 2.4–4.4 and 8.0–9.2 the diameter of the germ-tubes was quite constantly 3.3 μ, while from 9.2 to 9.6 they were 2.2 μ. Branching occurred at all concentrations except pH 2.4, 4.0, 6.8, and 7.2; each tube had from one to four branches and in most cases the branching was correlated with rapid growth; this was especially true at pH 5.6 and 7.6.

TABLE VII

D. CONCENTRICA (D. MO.)

GERMINATION OF ASCOSPORES AT DIFFERENT HYDROGEN-ION CONCENTRATIONS. THE RESULTS REPRESENT A COUNT OF 1200 ASCOSPORES FOR EACH HYDROGEN-ION CONCENTRATION, WITH PFEFFER AGAR AS THE MEDIUM

pH	Percent germination after 24 hours				Character of germ-tube. Length in microns	Branching —No. per spore	Conidia produced after days		
	Experiment								
	1	2	3	Av.			16	24	29
2.4	10.0	4.0	22.0	12.0	58.5c	0	+		
2.8	12.0	13.0	14.0	13.0	53.5c	1–4	−	−	+
3.2	12.0	14.0	17.0	14.3	55.0a	1–2	−	−	−
3.6	18.0	20.0	19.0	19.0	191.6c	1–2	−	−	−
4.0	20.0	23.0	24.0	22.3	39.0a	0	−	−	−
4.4	21.0	32.0	25.0	26.3	260.0a	2–3	−	−	−
4.8	44.0	46.0	49.0	46.3	206.0a	2–3	+		
5.2	64.0	32.0	66.0	54.0	170.0c	2–4	+		
5.6	73.0	74.0	63.0	70.0	251.3c	1–2	+		
6.0	52.0	69.0	56.0	59.0	126.6b	1–2	−	−	−
6.4	41.0	59.0	35.0	45.0	224.0b	3–4	−	−	+
6.8	53.0	40.0	63.0	52.0	41.0c	0	+		
7.2	41.0	31.0	33.0	35.0	77.3a	0	−	−	−
7.6	47.0	38.0	50.0	45.0	325.0c	1–4	−	−	+
8.0	40.0	31.0	43.0	38.0	94.0a	1–2	−	−	−
8.4	31.0	29.0	30.0	30.0	60.1a	1–3	−	−	−
8.8	30.0	27.0	45.0	34.0	83.0b	2–3	−	−	−
9.2	45.0	51.0	51.0	49.0	74.0c	1–2	−	−	+
9.6	27.0	35.0	43.0	35.0	180.0a	1–2	−	+	

a = majority with one germ-tube.
b = majority with two germ-tubes.
c = equal number with one and two germ-tubes.
+ = conidia present.
− = conidia absent.

The cultures were examined for conidia at three periods, at the end of 16, 24, and 29 days. After these periods the mycelium had so completely grown over the cover-glasses that it was impossible to see conidia without resorting to the use of a stain, for which purpose cotton-blue in lacto-phenol was employed. The conidia, which take the blue stain, are then easily seen. For each hydrogen-ion concentration four preparations were made at each period in order to eliminate as far as possible any experimental error. In the most acid concentrations of pH 2.4 conidia were present after 16 days. After this same length of time conidia were present in cultures started at pH 4.8., 5.2, 5.6, and 6.8. At all other hydrogen-ion concentrations no conidia were

observed. When 24 and 29 days had elapsed conidia were found also at pH 9.6, and at 2.8, 6.4, 7.6, and 9.2 respectively. Thus it appears that within a certain acid range and within a certain alkaline range conidia will not develop. Of course the absence of conidia may have been due to some other factor than hydrogen-ion concentration. In a hanging-drop culture in which the medium is present in such small quantities the food supply might soon be exhausted by the mycelium rapidly covering the medium. In most cases the cultures did not dry out, due to the maximum amount of solution placed in the cell when it was made up and due to the thorough sealing by vaseline. Therefore, although food supply is a very important factor in the occurrence of conidia, hydrogen-ion concentration also plays a very important role in the production of conidia.

Daldinia vernicosa.—With the identical treatment received by the ascospores of *D. concentrica*, the ascospores of *D. vernicosa* after 24 hours gave a characteristically poor percentage of germination. The maximum germination of 3.5 per cent was reached at pH 6.4. At pH 4.0, 4.4, and 4.8 there was 3 per cent germination, and 2.5 per cent, the next highest, occurred at pH 6.0. At pH 6.8, 2 per cent germination, and at pH 7.6 and 8.8 only 1 per cent germination was obtained. Only .5 per cent more was secured at pH 9.2. At the remaining hydrogen-ion concentrations there was no germination.

After 48 hours the percentages increased in every hydrogen-ion concentration, with a maximum of 17 per cent at pH 4.8 and a minimum of 4 per cent at pH 2.4. At the extreme alkaline end of the series the ascospores were stimulated, since 15 per cent germination occurred. The same percentage occurred at pH 6.4. The hydrogen-ion range as seen in fig. 3, from 4.0 through 6.4, was definitely the most favorable for germination, although the extreme alkaline medium of pH 9.6 stimulated the ascospores to nearly maximum germination.

The ascospores at the two extremes, pH 2.4 through 3.6, and pH 8.0 through 9.6, after 24 hours were only very slightly swollen, even though germination did occur at 8.8 and 9.2. In the intervening hydrogen-ion concentrations the swelling was slightly more pronounced, but at no hydrogen-ion concentration did the

swelling approximate that of the ascospores subjected to heat previous to inoculation. One germ-tube was the most common, although two did occur in some cases. The diameter remained constant, 4.4 μ, throughout the series, but the length varied; the longest germ-tubes occurred at pH 4.4 where the average length was 33 μ; the shortest one, 15.5 μ, occurred at pH 6.0. Most of the germ-tubes were not branched after 24 hours, but

TABLE VIII

D. VERNICOSA (D. MO.)

GERMINATION OF ASCOSPORES AT DIFFERENT HYDROGEN-ION CONCENTRATIONS. THE RESULTS REPRESENT A COUNT OF 1200 SPORES FOR EACH HYDROGEN-ION CONCENTRATION, WITH PFEFFER AGAR AS THE MEDIUM

pH	Percent germination after 24 hours. Experiment				Character of germ-tube. Length in μ	Branching —No. per spore	Conidia produced after days		
	1	2	3	Av.			16	24	29
2.4	0.0	0.0	0.0	0.0	0	0	−	−	+
2.8	0.0	0.0	0.0	0.0	0	0	−	−	−
3.2	0.0	0.0	0.0	0.0	0	0.	−	−	−
3.6	0.0	0.0	0.0	0.0	0	0	−	−	+
4.0	4.0	2.0	3.0	3.0	22.0a	0.	−	−	−
4.4	3.0	3.0	3.0	3.0	33.0a	1–2	−	−	−
4.8	5.0	3.0	1.0	3.0	30.0a	2–4	−	−	−
5.2	0.0	0.0	0.0	0.0	0	0	−	+	
5.6	0.0	0.0	0.0	0.0	0	0	−	−	−
6.0	2.5	3.0	2.0	2.5	15.5a	0	−	−	−
6.4	4.0	3.5	3.0	3.5	17.0a	0	−	−	−
6.8	2.0	2.0	2.0	2.0	27.0a	0	−	−	−
7.2	0.0	0.0	0.0	0.0	0	0	−	+	
7.6	1.0	1.0	1.0	1.0	19.5a	0	−	−	+
8.0	0.0	0.0	0.0	0.0	0	0	−	−	−
8.4	0.0	0.0	0.0	0.0	0	0	−	−	−
8.8	2.0	0.0	1.0	1.0	22.0a	0	−	+	
9.2	3.0	1.5	0.0	1.5	24.0a	0	−	−	−
9.6	0.0	0.0	0.0	0.0	0	0	−	−	−

a = majority with one germ-tube.
\+ = conidia present.
− = no conidia.

at pH 4.4 and 4.8 where the longest tubes occurred, one to four short branches were noted. After 16 days no conidia were observed; after 24 days conidia were found in cultures of pH 5.2, 7.2, and 8.8; and after 29 days conidia were found at pH 2.4, 3.6, and 7.6.

It is clear from the above experiments that the hydrogen-ion

concentration of the medium is a factor in the germination of the ascospores of *Daldinia concentrica* and *D. vernicosa;* both of these species find most favorable conditions between pH 4.8 and 6.0, although the latter species was greatly stimulated at pH 9.6. Extreme acidity and alkalinity with the exception noted were not conducive to germination. In general this conclusion can be said to agree with Panisset ('29), although unfortunately it is impossible to correlate her results with those of the writer's, since Panisset's are not stated quantitatively. Furthermore, she has adopted an arbitrary method of expressing acidity and alkalinity and her medium was a sucrose solution without the addition of mineral salts. As she demonstrated, and the writer has observed, differences of the substratum give different results in germination. Yet in spite of the differences of method it is clear that the ascospores tolerate a wide range of acid and basic conditions.

FACTORS INFLUENCING THE GROWTH OF MYCELIUM

THE EFFECT OF LIGHT AND DARKNESS

The effect of light and darkness on fungi is usually studied in reference to the production of fruiting bodies, and a great deal of work has been done on some of the well-known groups of fungi, such as *Saprolegnia, Mucor, Pilobolus,* etc. As seen by Lendner ('96), spores of *Mucor flavidus* were produced only in light; *Mucor racemosus* developed sporangia in darkness but the spores reached maturity only in light. Many species of moulds were found to show an excessive elongation of the sporophores in darkness.

The influence of light upon vegetative and reproductive processes of several algae and fungi was noted by Klebs ('96), who observed that the appearance of zoospores occurred most frequently in diffuse light and darkness, but conjugation took place in light. The intensity and duration of the illumination were the factors in the influence. The blue-violet rays appeared to be the cause of the specific action of light in such instances. A few years later Klebs ('00) asserted that light exerted no influence; growth of the vegetative, as well as the reproductive, organs of many fungous forms was utterly uninfluenced by the radiation. The growth of the sporophores in many species occurred absolutely regardless of light and darkness. Transpiration and nutrition may mask or alter the effects of illumination.

The observations of Stameroff ('97) offer some important and accurate evidence upon the influence of light upon the growth of fungi. Vegetative hyphae of *Mucor* and *Saprolegnia* show the same rate of growth in light and in darkness, while light is found to retard the elongation of the sporangiophore of *Mucor*.

Bullot's ('97) observations demonstrated that the growth of the sporangiophores of *Phycomyces* and *Mucor* was accelerated by diffuse light, thus substantiating the results of previous workers. It has been demonstrated by Grantz ('98) that *Pilobolus* formed sterile sporangiophores in darkness, but the etiolated sporangiophores might produce spores if given only 15 minutes exposure to light. He suggested that the etiolation phenomena of fungi are in fact reactions to the specific stimulations of light and darkness, and that various correlations are exhibited in these reactions.

In ascertaining the factors which determine the formation of pycnidia in *Plenodomus fuscomaculans*, Coons ('16) claims that, regardless of the media used, light is necessary for the formation of pycnidia. The process of the fruiting body formation is one which depends upon the metabolism of the fungus, and the direct result of the presence of light is oxidation, which may be brought about more rapidly by a higher temperature or richer food, in turn bringing about a more rapid metabolism. Thus the increased reproduction would be traced to the effect of oxidation. To confirm this, Leonian ('24) has shown the value of the presence of light as an oxidizing agent in the formation of pycnidia in some of the *Sphaeropsidales*. Occasionally a higher temperature not only replaced the effect of light, but became a more efficient agent for the promotion of fruiting. This was shown by subjecting cultures to light and darkness at room temperatures, then darkness at a constant temperature of 30° C. and light for 24 hours, then dark for 24 hours at 8° C. The effect of light may be traced to some series of photochemical changes which result in the inception of reproductive bodies. The aerial-branch systems have the potentiality of developing into reproductive structures before they receive energy from light. This is explanatory of degenerative structures which arise in darkness in equivalent positions to apothecia in normal cultures. The

relation of light to reproductive activity in this fungus thus operates relatively late upon regions of mycelium where the potentiality for development in a definite direction has already been determined. The absorption of a certain amount of energy from light is therefore a final phase in the sequence of causation concerned in the development. There are other factors which are concerned in this problem but one of the important ones is the presence of enzymes. Blackening observed in the absence of light in this case is said to be due to the abnormal effects of oxidizing enzymes, but light will inhibit these abnormal effects.

In the writer's experiment, the influence of light and darkness was regarded in relation to growth of the mycelium and not to the production of fruiting bodies, as the factors concerned in the formation of the perfect stage of *Daldinia* will be treated in a later paper. Since darkness proved to be more favorable to the germination of ascospores, its influence checked against that of light was tried here.

The source of inocula was mycelium from single ascospore cultures of *D. concentrica* (D. Mo.) (5 months old) and from mycelium developed from small bits of stromatic material in the case of *Daldinia Y* (B. S. D.) and *D. vernicosa* (C. Mass.), all of which were grown on oatmeal agar. Approximately one square centimeter of actively growing mycelium of the same age in each species served as the inocula. Four Erlenmeyer flasks of 250-cc. capacity with about 100 cc. of oatmeal or Pfeffer agar were made up for each species. Two were placed in the light of a north window at room temperature and two were put into a thoroughly darkened cabinet which was approximately at room temperature. The cultures were examined after 5, 10, 12, and 30 days.

Daldinia concentrica.—On oatmeal agar the cultures in the dark developed a much more aerial, denser, and more abundant mycelium than the ones in the light. The mycelium grew about twice as rapidly in the former as in the latter. The tufts of mycelium in both cases were small, at first white, but after 30 days the one in the light was noted to have changed to a "Pallid Mauve Gray,"[1] then to a "Mouse Gray" in the lightest portions,

[1] Ridgway, R. Color standards and color nomenclature. Washington, D. C. 1912, used where colors are in quotation marks.

and finally to a "Quaker Drab" in the darkest portions. Where coming in contact with the glass the mycelium became blackened. In the dark the mycelium underwent changes of color, but in a very much milder degree. No blackening was noted, and it was therefore supposed that continuous light was necessary. The short exposures to light when the cultures were examined seemed to be sufficient stimulation to cause changes from white to "Pallid Mauve Gray." Later, cultures of *D. concentrica* were put into the dark and not examined for 30 days, after which time the mycelium had changed very little from white to a "Pale Dull Gray." The medium did not change appreciably in color, except for the slight darkening due to the blackened mycelium in contact with the glass.

Zonation was observed in the cultures kept in the light and darkness, but it was more pronounced in the former. Conidia in the cultures of this species, as well as of *D. vernicosa*, as far as the writer has observed, are usually associated with the presence of zones. The zones are apparently correlated with the rate of growth, for in the light, where the mycelium grows less rapidly than in the darkness, the zones are more distinct. It would seem then that the zones are formed as the result of staling products, and the check in growth induces the formation of conidia on a favorable medium.

On Pfeffer agar the mycelium responded in a similar way to light and darkness. The only difference was noted in the characteristic delicacy and sparseness of growth, due to the lack of carbohydrates in the medium.

Daldinia Y.—Under the same conditions as *D. concentrica* the mycelium of this species grew less rapidly at first, but after ten days the growth proceeded rapidly and at the end of the experiment far exceeded the quantity and extent of mycelium of *D. concentrica*. After 5 days the radial growth of the mycelium in the dark was about .7 cm., was dense and cottony, while in the light the growth was .5 cm., and was more delicate and appressed. After 10 days the mycelium in the dark had a radial growth of about 6.5 cm. and was very dense and cottony, while in the light the radial growth was only about .5 cm. less, but the mycelium was prostrate and began to take on a cottony appearance due to

its increasing abundance. The mycelium of cultures kept in the dark had covered the surface of the agar after 12 days, and a ring of 11 small turbinate tufts of mycelium and one tuft at the place of inoculation gave the culture a very nodular appearance. In cultures exposed to light the mycelium had also covered the surface of the medium, and two large fused turbinate tufts of mycelium were noted at the place of inoculation, while several smaller less distinct tufts were scattered over the surface of the medium. After 30 days the cultures in the dark had developed further and the large turbinate tufts of mycelium strongly resembled ascocarps (pl. 36, fig. 2). The mycelium had grown up on the sides of the flask and under the medium and had become a "Light Buff" color. In the light the mycelium was a "Vinaceous Buff" color; it had grown less rapidly than in the dark and had not grown up on the sides of the flask or under the medium. A secretion, dilute "Claret Brown," oozed out from the medium and collected in small droplets over the surface of the mycelium in cultures maintained in the dark and only after two months of growth.

A similar series of experiments on the effect of light and darkness, with Pfeffer agar as the medium, was carried out. Here again this medium proved to be a poor one for mycelial development, but the different effects of light and darkness on the growth were equally evident. In both cases the mycelium was delicate, although in the dark it was slightly denser, more aerial, and grew about one and one-half times more rapidly than in cultures exposed to light. Zonation was more definite in the light than in the dark.

Daldinia vernicosa (C. Mass.).—With the same treatment as the two preceding species, the mycelium of *D. vernicosa* on oatmeal agar grew very luxuriantly; darkness was more conducive to rapid and dense growth of mycelium than light. In the dark the mycelium was densely cottony, while in the light it was more delicate, sparse and uneven, at first white, then after 30 days a "Pale Pinkish Buff," and the medium had turned a "Hazel" color. A dilute "Claret Brown" secretion oozed out from the medium and collected in small droplets over the surface of the mycelium which by this time had become leathery. The secretion

was also noted in the culture in the dark but in the light there was a greater quantity.

On Pfeffer agar, the mycelium responded in a similar manner to that on oatmeal agar when exposed to light and darkness, except the growth was less abundant and more delicate.

Although this experiment was undertaken primarily with further work in mind to determine under what conditions of light the mycelium grew more favorably, results have been so illustrative that they are considered a part of this study. Thus the results are in agreement with those of the investigators who have found that the mycelium of certain fungi grow more rapidly in the dark than in the light. Light does retard growth, as some workers have demonstrated with other fungi. No doubt the medium plays a part in the growth response, as is seen in the cultures where Pfeffer agar was used, but the cultures in the light and dark were distinct on both oatmeal and Pfeffer agars in all three species. In general, the mycelium in the dark grew more rapidly, more upright and more evenly than in the light. The great abundance of growth in both light and darkness was characteristic of *Daldinia Y*. The heavier growth of mycelium was probably not the result of the use of stromatic material, since even from single ascospore cultures of *D. vernicosa* the mycelium was almost as abundant as in the case of *Daldinia Y* derived from stromatic material. Still further proof of this contention is found in sprayed ascospore cultures of *D. concentrica* which were allowed to develop mycelium from hundreds of ascospores, and the volume of growth never attained that of *Daldinia Y*. Next to *Daldinia Y*, *Daldinia vernicosa* developed the most abundant mycelium, whether from a single ascospore or from a bit of stromatic material. Although the ascocarp of *Daldinia Y* and *D. concentrica* closely resemble one another, in behavior and appearance the former species in culture more closely resembles *D. vernicosa*.

The "Claret Brown" secretion of *D. vernicosa*, it will be remembered, developed in cultures exposed both to light and to darkness after a month, but in the case of *Daldinia Y*, only after 2 months in the dark. The secretions were not observed in the cultures where Pfeffer agar was used. It might be deduced that the secretion develops only on a medium rich in nutrient materials.

That this may very likely be true is suggested by the fact that *Avena sativa* grain (according to Wehmer) contains about 50–60 per cent starch and 2–5 per cent sucrose and dextrin. On the other hand, the secretion may be associated with the staling of the culture and may be a metabolic product of the fungus formed as a result of age or moisture. It has been stated that the direct result of the presence of light may be oxidation, and the photochemical changes might account for the presence of the secretion, were it not for the fact that the secretion appears in the dark as well as in the light.

The blackening of the mycelium of *D. concentrica* in contact with the glass was observed in month-old cultures in the light, but not in the dark. Very little color change of the mycelium occurred when the cultures were in continuous darkness, although in a parallel series very short exposures (not exceeding five minutes) at several intervals resulted in distinct changes. Thus it appears that the blackening is due to an oxidation process which is probably stimulated by light. Blackening never occurs in *Daldinia Y* or in *D. vernicosa*.

Conidia were produced by all three species irrespective of whether the cultures were maintained in darkness or light, yet in the light they were more abundant, especially in the denser parts of the zone.

THE EFFECT OF DIFFERENT MEDIA AND DIFFERENT TEMPERATURES

The nutrition of the fungi has for years been a subject demanding the attention of many investigators, but parasitic forms have engaged interest more than saprophytic forms, because of their economic importance. The sources of carbon and nitrogen have been varied in all sorts of media formulae and in most cases have proved to be entirely specific in their requirements. Webb ('21) found that the fungi which he used did not grow as well in a medium where cellulose was a source of carbon as where the sugars and peptones were a source. Organic forms of nitrogen were superior to inorganic forms. Wolpert ('24) states that monobasic salts are better for fungous growth, while di- and tribasic salts, since they are alkaline, inhibit the growth of mycelium. Brown ('23), who studied six strains of *Fusarium*, found that by

increasing the glucose in the medium, more aerial mycelium developed, and with an increase in the phosphate form of neutral salts and in starch, sporulation was stimulated while aerial mycelium was inhibited. With an acid phosphate the results were the opposite. Color in the medium was found to be due to a high carbon and nitrogen ratio together with a low phosphate concentration.

Another important factor in the development of fungi is temperature. The combined effect of substratum and temperature is in all probability a factor in mycelium development of many fungi which cannot be neglected. The following experiment shows that this is true for members of this genus.

As a source of heat, constant-temperature ovens of 23–24° C., 27–28° C., and 32–33° C. were employed. Triplicate cultures were made in petri dishes with *Platanus* decoction, Leonian, Pfeffer, and oatmeal agars as the media. The experiment was performed two different times, and an average of these was calculated. The plates were inoculated in the usual manner with mycelium developed from single-spore cultures of *D. concentrica* (D. Mo.) and *D. vernicosa* (D. Mo.) (both five months old), but the mycelium of *Daldinia Y* (B. S. D.) was developed from small bits of stromatic material. Readings were taken after 48, 120, 168, and 216 hours, then the rate of growth per day was calculated at 120 hours in the case of *D. concentrica* and *Daldinia Y*, and at 48 hours in the case of both *D. vernicosa*, since by these two periods the mycelium had almost covered the surface of the medium. The results are given in tables IX, X, XI, and XII.

Daldinia concentrica.—On *Platanus* decoction agar most rapid growth, 15.66 mm. per day, occurred at 27° C.; slightly less rapid growth, 12.33 mm. per day, at 23° C.; while the least occurred at 32° C., with 11.00 mm. per day. At all temperatures the growth was even but delicate, although at 23° C. the mycelium was at first aerial but became prostrate at the end of 48 hours. At the two higher temperatures the colonies were prostrate from the beginning.

The most rapid rate of growth per day, 12.33 mm., on Leonian agar, was obtained at 23° C. Here 27° C. was second best with 11.66 mm. growth per day, and the growth seemed to be rather

slow at 32° C., with only 5.33 mm. per day. On this medium at all temperatures the mycelium was aerial, and the growth was even, fine, but dense. At all temperatures one zone appeared after 120 hours, but at 32° C. an additional zone appeared by the end of 168 hours. Zones in these cases indicated the presence of conidia, which were present in all cultures at 120 hours.

On Pfeffer agar the mycelium grew fairly rapidly, but it was extremely delicate. The most rapid rate of growth per day, 13.33 mm., was secured at 27° C.; at 23° C. the next highest of 10.00 mm. occurred; while at 32° C. the mycelium was least extensive or 7.66 mm. per day. The mycelium on this medium at 23° C. was characteristically appressed after 48 hours, although until 24 hours it had been aerial. At higher temperatures it was at first prostrate, but then became appressed. The growth of mycelium at 23° C. and 27° C. was uneven, although at 32° C. it was even. Delicate, fuzzy mycelium was not confined to any one temperature. Conidia were produced only at 32° C. after 124 hours.

TABLE IX

D. CONCENTRICA (D. MO.)

THE EFFECT OF DIFFERENT MEDIA AND DIFFERENT TEMPERATURES ON RADIAL GROWTH OF MYCELIUM

Medium	Temp. ° C.	Mm. growth after hours				Rate of growth per day at 120 hrs. in mm.
		48	120	168	216	
Platanus decoction agar	23	20	57	PC	PC	12.33
	27	22	69	PC	PC	15.66
	32	18	51	72	PC	11.00
Leonian agar	23	20	57*	PC	PC	12.33
	27	15	50*	PC	PC	11.66
	32	14	30*	50	PC	5.33
Pfeffer agar	23	8	38	52	PC	10.00
	27	14	54	PC	PC	13.33
	32	24	47*	PC	PC	7.66
Oatmeal agar	23	16	48	61	PC	10.66
	27	11	27*	47	60	5.33
	32	12	23*	28	34	3.66

PC = plate covered
* = conidia present.

Oatmeal agar, which has usually proved to be the best medium for mycelial growth, here actually proved to be a medium on

which the mycelium grew more slowly than any of the other media tried. Although the rate of growth was slow, more luxuriant and dense mycelium was produced. The greatest rate of growth per day, 10.66 mm., was secured at the temperature nearest that of room temperature, 23° C.; at 27° C. growth was 5.33 mm., and at 32° C. was only 3.66 mm. per day. On this medium, as was characteristic under all conditions tried, the mycelium was aerial. After only 24 hours, the growth of the mycelium was even, very dense and fluffy, while after 120 hours the nodular character of the mycelium had become evident, and at all temperatures there were no zones present, but at 27° and at 32° C. conidia were observed.

Daldinia Y.—The three temperatures seemed to be almost equally favorable for the growth of the mycelium of *Daldinia Y* on *Platanus* decoction agar. There was a slightly better growth at 23° C. than at the other temperatures, but the rate of growth was rapid, 10.33 mm. per day. This medium which has proved to be a rather poor one for profuse development of mycelium, was not conducive to aerial mycelium. At 23° C. and 27° C. the growth was even, but at 32° C. it was uneven, and at all three temperatures the mycelium was prostrate, very delicate, azonate, and no conidia were present.

On Leonian agar the rate of growth was rapid, for at 27° C. the surface of the agar was covered after 120 hours, and 32° C. was favorable as shown by the 13.33 mm. of growth per day. Only 7.33 mm. growth per day was obtained at 23° C. At the highest temperature the mycelium was prostrate after 24 hours, then slightly aerial, but at 23° C. and 27° C. it was aerial from the beginning. In spite of the fact that this medium was favorable to growth the mycelium was delicate, uneven, and azonate at 23° C.; at 27° C. it was abundant but fine. At the highest temperature after 48 hours, it was delicate and fuzzy, but later became smooth and appressed. Conidia were present only in cultures maintained at 32° C. after 120 hours.

If rapid rate of growth of mycelium were a criterion for a good medium, Pfeffer agar might be considered one, for at 23° C. 12.66 mm. growth per day. and at 32° C. 13.33 mm. of growth was obtained, but as regards quantity of growth, Leonian and oat-

meal agars are far superior. The rate of growth on Pfeffer agar was rapid, but extremely delicate and neither zones nor conidia were present at any of the temperatures.

On oatmeal agar the slowest rate of growth, 8.33 mm., was observed at 23° C., and at 27° and 32° C. the same rate, 1.33 mm., was secured. The mycelium was aerial, though, even after 24 hours when the nodular character began to develop, and the mycelium became very dense and fluffy. Conidia were present at 23° C. after 168 hours and at 27° C. after 120 hours, although there were no zones.

TABLE X

DALDINIA Y (B. S. D.)

THE EFFECT OF DIFFERENT MEDIA AND DIFFERENT TEMPERATURES ON RADIAL GROWTH OF MYCELIUM

Medium	Temp. ° C.	Mm. of growth after hours				Rate of growth per day at 120 hrs. in mm.
		48	120	168	216	
Platanus decoction agar	23	2	33	55	PC	10.33
	27	4	34	PC	PCS	10.00
	32	5	35	PC	PCS	10.00
Leonian agar	23	2	24	49	PC	7.33
	27	20	PC	PCS	PCS	
	32	4	43*	PCS	PCS	13.33
Pfeffer agar	23	2	40	PC	PCS	12.66
	27	2	30	PC	PCS	9.33
	32	0	40	75	PC	13.33
Oatmeal agar	23	1	26	67*	PC	8.33
	27	16	20*	60	PC	1.33
	32	0	4	6	8	1.33

* = conidia present.
PC = plate covered.
PCS = plate and sides covered.

Daldinia vernicosa (D. Mo.).—In the two specimens of *D. vernicosa* the rate of growth exceeded that of the preceding species, and it was necessary to calculate the radial rate of growth per day after 48 hours. On *Platanus* agar the highest temperature seemed most favorable to rapid growth, for 15.5 mm. was secured, while at 27° C. the rate was only .5 mm. less; but at 23° C. it was considerably less, only 9.5 mm. per day. At 23° C. the mycelium was at first aerial, but after 48 hours it was prostrate. This was not the case at higher temperatures, for the mycelium was aerial.

Delicate, fuzzy, and uneven growth was characteristic at all the temperatures, and no conidia and no zones were formed.

A very rapid rate of growth, 16.5 mm. per day, occurred on Leonian agar at 32° C. The next highest temperature of 27° C. gave more rapid growth, 12.5 mm., than 23° C. where 11.0 mm. per day was secured. At all of the temperatures the mycelium was aerial except at 32° C. where at first it was prostrate, later becoming aerial. At 23° C. the mycelium was even and dense, while at higher temperatures it was uneven, delicate, and fuzzy. Even though no zones were formed at any of the temperatures, conidia were present at 23° C. after 120 hours, and not in cultures kept at 27° C. or 32° C.

TABLE XI

D. VERNICOSA (D. MO.)

THE EFFECT OF DIFFERENT MEDIA AND DIFFERENT TEMPERATURES ON RADIAL GROWTH OF MYCELIUM

Medium	Temp. ° C.	Mm. of growth after hours				Rate of growth per day at 48 hrs. in mm.
		48	120	168	216	
Platanus decoction agar	23	19	PC	PCHS	PCS	9.5
	27	30	95	PCHS	PCS	15.0
	32	31	PC	PCHS	PCS	15.5
Leonian agar	23	22	PC*	PCS	PCS	11.0
	27	25	PCS	PCS	PCS	12.5
	32	33	97	PCS	PCS	16.5
Pfeffer agar	23	30	PC	PCHS	PCS	15.0
	27	20	PC	PCHS	PCS	10.0
	32	17	PC	PCS	PCS	8.5
Oatmeal agar	23	13	55	PCHS	PCS	6.5
	27	17	41*	PCS	PCS	8.5
	32	20	35	68	PC	10.0

* = conidia present. PC = plate covered. PCS = plate and sides covered. PCHS = plate and half of sides covered.

On Pfeffer agar at 23° C. the rapid rate of growth of 15.0 mm. was secured, while at 27° C. 10 mm., and at 32° C. only 8.5 mm. of radial growth per day developed. After 48 hours the mycelium at 23° and at 27° C. was aerial, although it was appressed at first. At 32° C. it remained appressed.

At 32° C. 10 mm. of growth per day was observed to develop on oatmeal agar, while at 23° C. 6.5 mm., and at 27° C. 8.5 mm.

was obtained. Here again, oatmeal seemed least favorable to rapid growth, but the abundance of growth on this medium exceeded that on other media. It was typically aerial, at first even, then fluffy and nodular. At 23° and 27° C. it was azonate, but at 32° C. two zones were noted after 120 hours, and at 23° C. and 32° C. no conidia were noted, but at 27° C. after 120 hours conidia were present.

Daldinia vernicosa (C. Mass.).—At the three temperatures on *Platanus* decoction agar, the rates of growth per day were only .5 mm. apart, with the maximum of 11.5 at 23° C. At this temperature the mycelium was at first aerial, but after 48 hours, prostrate. At the two higher temperatures the mycelium was aerial. Even growth after 120 hours was characteristic of cultures maintained at 23° C. but at higher temperatures the growth was uneven. It was delicate, azonate, and no conidia were present.

TABLE XII

D. VERNICOSA (C. MASS.)

THE EFFECT OF DIFFERENT MEDIA AND DIFFERENT TEMPERATURES ON RADIAL GROWTH OF MYCELIUM

Medium	Temp. ° C.	Mm. growth after hours				Rate of growth per day at 48 hrs. in mm.
		48	120	168	216	
Platanus decoction agar	23	23	PC	PCS	PCS	11.5
	27	20	PC	PCHS	PCS	10.0
	32	21	PC	PCS	PCS	10.5
Leonian agar	23	17	PC	PCS	PCS	8.5
	27	29	PCS	PCS	PCS	14.5
	32	40	PCS	PCS	PCS	20.0
Pfeffer agar	23	14	PC	PCS	PCS	7.0
	27	16	PC	PCS	PCS	8.0
	32	33	PC	PCS	PCS	16.5
Oatmeal agar	23	4	25*	42	PC	2.0
	27	15	96*	PCS	PCS	7.5
	32	9	27*	52	PC	4.5

* = conidia present.
PC = plate covered.
PCS = plate and sides covered
PCHS = plate and half of sides covered.

A most rapid growth of mycelium was observed on Leonian agar, where 20.0 mm. per day developed at 32° C., while at 27° C. 14.5 mm., and at 23° C. only 8.5 mm. growth was obtained. Un-

even, aerial mycelium was secured at all temperatures, and while it was delicate and sparse at 23° C. at higher temperatures it was dense and fluffy. There were neither zones nor conidia.

The second highest rate of growth in this whole series was obtained on Pfeffer agar at 32° C. where 16.5 mm. of growth was secured. Only 7.0 and 8.0 mm. were secured at 23° and 27° C. respectively. Where the most rapid rate of growth occurred the mycelium was extremely delicate, uneven, and appressed; at 23° and at 27° C. it was aerial, even, the former delicate, the latter rather dense and fluffy at the outermost margin after 168 hours. In the three series the colonies were azonate and produced no conidia.

On oatmeal agar only 2.0, 7.5, and 4.5 mm. of growth per day was obtained at the temperatures in the ascending order of their intensities. Aerial, dense, and fluffy mycelium was noted at all temperatures, but the evenness became broken by nodular tufts after 120 hours when conidia occurred at the three temperatures. The higher temperatures induced zones to appear; at 23° C. there were two zones.

The close physiological relationship of *Daldinia Y* and *D. vernicosa* is indicated by the fact that these two species grow most rapidly on Leonian agar at 32° C., whereas the mycelium of *D. concentrica* responds most favorably on *Platanus* agar at 27° C. On the other hand, all three species produce the most abundant growth (as contrasted with the most rapid rate of growth) on oatmeal agar.

The nutrient values of the media employed in this study have been discussed above but there is an additional one here, oatmeal, which has not been previously described. The oatmeal, which was used in the preparation of the medium, was finely ground, then prepared in the usual manner. According to Wehmer ('11) the average percentage mineral content of *Avena sativa* grains is as follows: 3.2 ash, 30–40 SiO_2, 23–30 P_2O_5, 15–20 K_2O, 5–7 MgO, 2–4 CaO, 1–2.5 SO_3, minute amounts of Na_2O, and Cl and Cu. The analysis of the grain varies a great deal, but the percentages of the constituents given are: 12.8 H_2O, 10.25 nitrogenous substances, 5.27 fats, 59.68 nitrogen-free extractable material, 9.97 rough fiber, 3.02 ash, about 50–60 starch, and 2–5 sugar (sucrose)

and dextrin. Thus from the large quantity of carbohydrates, one may well see why the mycelium was so dense and fluffy in all of the species. It will be noted that where carbohydrates are abundant, the mycelium is aerial and becomes very nodular in the case of *D. vernicosa* and *Daldinia Y*, but very much less pronounced in *D. concentrica*. Another character which appears to be confined to oatmeal agar is the presence of conidia, for more conidia occurred on this medium than on any other. Since the two higher temperatures seem to be slightly more favorable than 23° C. the acceleration of the chemical activities by the higher temperatures may make it possible for the fungi to utilize carbohydrates more readily. Furthermore, it is possible that, aside from the carbohydrate, the nitrogen content, and the factor of temperature, the possible presence of vitamins in the oatmeal contributes to the luxuriant and abundant growth of mycelium. Growth is characteristically azonate at the higher temperatures and at the same time conidia are formed. It has been said of some fungi that zonation may be either induced by temperature or the proper food, but neither causes zonation here, evidently signifying that staling products are not localized concentrically.

The high carbohydrate content of Leonian agar is conducive to aerial and rather dense mycelium in all species. With one exception, *D. concentrica*, no zones were produced, but more conidia were observed on this medium than on any other except oatmeal agar.

Since Pfeffer agar contains no sugars, the growth of mycelium in all cases was very delicate, usually appressed and azonate with the exception of *D. concentrica*, where one zone and conidia were found. Evidently the abundance but not the rate of growth is dependent upon carbohydrates, as far as could be learned from petri dish cultures.

It is not surprising to find that *D. concentrica* grows more rapidly on *Platanus* decoction agar than any of the other species, for it will be remembered that this species in Missouri usually occurs on this host. It was surprising, however, to find that there were no conidia, even though two rather distinct zones were present.

Daldinia Y produces mycelium very similar in rate of growth, texture, and quantity to that of *D. vernicosa*, although the asco-

carp and ascospores are obviously closely related to *D. concentrica*. The behavior of *D. vernicosa* from the two different localities was very similar, as is seen by the two most rapid rates of growth that were obtained on Leonian agar at 32° C. Other characters were similar; dissimilarities were due probably to the differences in age and habitat. As a whole, 23° and 27° C. seemed to be most favorable for rapid growth of *D. concentrica* and its near relative, while for *D. vernicosa* 32° C. was more conducive to rapid growth.

Thus from these results it is demonstrated that the combined effect of medium and temperature upon mycelial development is of utmost significance in a comparative study of this kind. Such observations substantiate the statement of Coons, that: "The development of an organism is the resultant of the environment working upon definite internal potentialities of the organism and that with a given potentiality, the same external conditions call forth the same response with the constance of a chemical reaction."

THE EFFECT OF DIFFERENT HYDROGEN-ION CONCENTRATIONS ON THE RADIAL GROWTH OF MYCELIUM

Within the last few years no physiologist has considered that he has run the gamut of factors in any investigation until he has tested the relative acidity or alkalinity of the organism or the medium on which the organism grows. Especially for parasitic fungi, the hydrogen-ion concentration of the substratum has proved to be a factor in growth and development. Usually a slightly acid medium is favorable for the germination of spores, while for mycelial growth slight alkalinity is more favorable. In many cases, though, fungi have shown their ability to grow over wide ranges of hydrogen-ion concentrations and become adapted to a particular concentration if it is not absolutely toxic. MacInnes ('22) has briefly summarized the influence of hydrogen-ion concentration on many fungi, and she has demonstrated that the wheat scab organism is capable of growing over a wide range of hydrogen-ion concentrations.

In this experiment mycelium from single ascospore cultures of five-months-old material of *D. concentrica* (D. Mo.), *D. vernicosa* (D. Mo.), and *Daldinia X* (V. P. Mo.) was used. All of the asco-

TABLE XIII

THE EFFECT OF DIFFERENT HYDROGEN-ION CONCENTRATIONS ON THE RADIAL GROWTH OF MYCELIUM

pH	*D. concentrica* (D. Mo.)			*D. vernicosa* (D. Mo.)				*Daldinia X* (V. P. Mo.)		
	Millimeter growth in hours.									
	48	120	per day after 120	48	72	120	per day after 72	48	120	per day after 120
3.0	0	0	0	7.5	13.0	PC	1.88	.5	5.0	.9
3.4	0	0	0	10.5	25.0	PC	4.88	.5	7.0*	1.3
3.8	.5	10.0	1.9	15.5	28.0	PC	4.11	.5	17.0*	3.3
4.2	1.5	12.0	2.1	15.0	33.0	PC	6.00	2.0	20.5*	3.7
4.6	2.5	18.0	3.1	15.5	33.0	PC	5.88	2.5	27.0	4.9
5.0	2.5	16.5*	2.8	13.5	35.0	PC	7.11	2.5	30.0	5.5
5.4	2.5	17.5	3.0	14.5	34.0	PC	6.55	3.0	34.0	6.2
5.8	2.5	15.5*	2.6	13.5	34.0	PC	6.88	3.5	29.0	5.1
6.2	2.5	18.5*	3.2	14.0	33.0	PC	6.33	5.0	34.0	5.8
6.6	2.0	19.5*	3.5	11.5	24.0	PC	4.11	3.5	30.0	5.3
7.0	1.5	15.5	2.8	9.5	22.0	PC	4.11	5.0	31.0	5.2
7.4	1.5	12.0	2.1	6.0	20.0	PC	4.66	3.0	25.0	4.4
7.8	.5	.5	0	1.5	12.0	16.5	3.5	2.0	13.5*	2.3

* = conidia present. PC = plate covered.

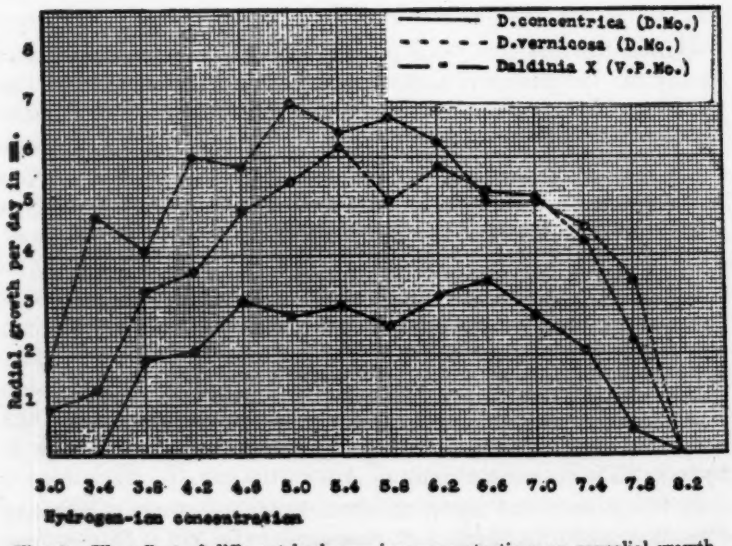

Fig. 4. The effect of different hydrogen-ion concentrations on mycelial growth.

spores had been frozen at −5° C. for one and one-half months, then sprayed on Pfeffer solution agar, and single germinated ascospores were cut out and put on oatmeal-agar plates. Approximately same sizes, ¾–1 sq. cm., of actively growing mycelium were used as inocula, all of which were from stock cultures made up at the same time. Two different experiments were run and quadruple sets of each hydrogen-ion concentration were made, then an average taken. The range was from pH 3.0 through 7.8; at first pH 8.6 was included, but when no growth was obtained at this concentration it was omitted in the second experiment. Leonian agar was used and adjusted by citric acid and by NaOH. After inoculation the cultures were put into a darkened cabinet and maintained at room temperature. The results are given in table XIII, and the calculated radial growth per day is given in fig. 4 (see plate 36, figs. 3–5).

Daldinia concentrica.—Such extreme acid media as pH 3.0 and 3.4 are seen to be toxic to the mycelium of *D. concentrica*, for no growth was obtained at these two concentrations. The most rapid rate of growth was obtained at pH 6.6, where 3.5 mm. of growth per day was secured, and the slowest rate of 1.9 mm. was obtained at pH 3.8. Thus it seems that near neutrality is most favorable for rapid rate of growth. At pH 3.8 and 4.2 and 4.6, the mycelium was uneven, delicate, and rather prostrate; although one zone appeared at pH 4.6 after 120 hours, no conidia were present. The mycelium at pH 5.0 and 5.4 was even but prostrate and delicate; the former produced two zones and conidia after 120 hours, while the latter produced no conidia and was azonate. From pH 5.8 through 7.0, the mycelium was aerial, even, and rather dense; at pH 6.6 it was nodular, very dense, and fluffy, and conidia were present, but the colonies were azonate. At pH 6.2 the mycelium was not quite so fluffy, but in this case conidia were present. At pH 7.0, 7.4, and 7.8 the mycelium was uneven, delicate, and fuzzy; at the first one it was appressed and on the other two rather prostrate.

Daldinia vernicosa.—*D. vernicosa* not only grows rapidly but produces abundant mycelium. It was necessary to calculate the growth per day after 72 hours, since by 120 hours it had covered the plate. The minimum rate per day occurred at pH 3.0 where 1.88

mm. was obtained, while the maximum occurred at pH 5.0 where there was 7.11 mm. growth per day. As a whole the range from 4.2 through 6.2 was most favorable for rapid growth. From pH 3.0 through 4.2 the mycelium was appressed, even, and delicate; at pH 4.6, prostrate, and even, except for the outermost margin which was aerial and fluffy; from pH 5.0 through 6.2 it was characteristically aerial, even, and rather dense, except at pH 5.0 where it was less dense. At the remaining hydrogen-ion concentrations the mycelium was appressed, uneven, delicate, and fuzzy; at pH 7.8 it was very sparse. At no hydrogen-ion concentration did conidia or zones appear.

Daldinia X.—The mycelium of *Daldinia X* grew through the entire range of hydrogen-ion concentrations, but very slowly at pH 3.0 where only .9 mm. per day was calculated; the maximum occurred at pH 5.4 where 6.2 mm. of growth per day was observed. The optimum range was evidently from pH 5.4 through 7.0. At the first two concentrations the mycelium was appressed, fuzzy, and very delicate, but even. Conidia were present on pH 3.4 but not 3.0. On pH 3.8 and 4.2 the mycelium was even, rather aerial, dense, and fluffy; conidia were present in both cases. The mycelium was aerial, delicate, and fuzzy from pH 4.6 through 6.2, but rather dense and fluffy at pH 6.6, 7.0, and 7.4. On the most alkaline medium the mycelium was delicate and fuzzy with but a few conidia. There were from four to five zones after 72 hours on pH 6.2, 6.6, and 7.0, but no conidia. On pH 5.0 and 5.4 there were four and three zones respectively after the same length of time, but here, as in the preceding cases, no conidia were observed.

Thus compared, the differences in the three species are clearly distinct. The most abundant growth in the case of *D. concentrica* occurred within the narrow limits defined by pH 6.2 and 6.6; in the case of *D. vernicosa* at pH 5.4, 5.8, and 6.2, and in the case of *Daldinia X*, over a less acid range, from pH 6.6 through 7.4. Although the ascocarp and ascospores of *Daldinia X* and *D. vernicosa* are similar, the mycelium on these different hydrogen-ion concentrations looks very much more like *D. concentrica* than *D. vernicosa*. Another similarity is observed between *Daldinia X* and *D. concentrica* in the presence of conidia after 120 hours; the range in which they occurred was different, but in *D. vernicosa* they did not occur at all within this time limit of the experiment.

Brief Morphological Characters of the Ascocarp and Asexual Stage of the Forms Studied

In view of the physiological behavior of the forms it seems desirable to give at least a brief and superficial account of the gross morphology, since the physiological reactions of the fungi are so clearly correlated with the morphological characters. The

	D. concentrica	*Daldinia Y*	*D. vernicosa*	*Daldinia X*
Ascocarp				
External color	"Hays Brown" to "Dark Vinaceous Brown"	Dull black to bronzed black	"Hays Brown," becoming black and strongly laccate	"Snuff" to "Verona Brown"
Surface	Moderately papillate to base	Prominently papillate to base	Minutely papillate half way to base	Minutely reticulate on top. Zones prominent on stipe
Shape and size	Single or confluent, hemispherical. Size variable, 1–6 cm. diam.	Single, hemispherical, 2–4 cm. diam.	Usually single, substipitate, spherical, clavate, 1–3 cm. diam. above stipe	Confluent or single, substipitate in cross-section, elongate-elliptical or circular in young forms, .5–1.5 cm. diam.
Internal color	"Drab Gray" zones alternating with "Bone Brown"	"Benzo" zones alternating with "Fuscous"	Thin black zones alternating with wider "Pale Dull Gray"	Thin black zones alternating with wider "Pale Dull Gray"
Texture	Soft, persistent, and compact	Soft, persistent, and compact	Fragile, coalescing to produce irregular cavities, collapsing	Fragile, coalescing to produce irregular cavities, collapsing, more persistent than in *D. vernicosa*
Asexual				
Conidiophore size	3.2 μ diam.	2–2.2 μ diam.	2–2.2 μ diam.	4.8 μ diam.
Conidia shape and color	Singly or in whorls, ellipsoidal, hyaline	Singly or in whorls, ellipsoidal, hyaline	Singly or in whorls, ovoid to ellipsoidal, hyaline	Singly or in whorls, elongated, elliptical, hyaline
Size	2.4–3.2 x 4.8–6.4 μ	2–3 x 4–6 μ	2.4–3.2 x 2.4–3.2 μ	8–12.8 x 4.4 μ

descriptions are superficial, because more exact descriptions are to follow in a monographic study. The table is intended to emphasize the main differences only.

Summary

1. In these investigations, factors influencing the germination of ascospores and the development of mycelium of *D. concentrica*, *D. vernicosa*, and two unnamed species, *Daldinia X* and *Daldinia Y*, have been studied comparatively in pure culture.

2. It was found in most cases that ascospores over a year old failed to germinate either because of age or as a result of treatment with fumigants.

3. In experiments devised to determine the effects of oxygen, three sources were used: oxygen produced by the photosynthetic process of the alga, *Pleurococcus* sp., hydrogen-peroxide, and compressed oxygen. Germination of ascospores, both frozen and unfrozen of *D. concentrica*, was stimulated by oxygen from the first and last source. Ascospores of *D. vernicosa*, however, appeared to thrive better in the absence of oxygen artificially supplied. In both species, when germination occurred, growth of the germtubes appeared to be stimulated. Ascospores of *Daldinia X* were indifferent to the oxygen treatment.

4. Neither the ascospores of *D. concentrica* nor *D. vernicosa* germinated in pepsin solutions from .1 to 10 per cent.

5. Ultra-violet rays (578–289 μμ) definitely stimulated germination of both frozen and unfrozen ascospores of *D. concentrica*. Maximum germination of *D. vernicosa* (4 per cent) was secured from unrayed but frozen ascospores; raying slightly favored germination of unfrozen ascospores. Frozen and unfrozen ascospores of *Daldinia X* were adversely affected by raying.

6. Ascospores of *D. vernicosa* and *Daldinia X* frozen for 3 months at —5° C. were stimulated to increased germination, whereas the opposite effect resulted when those of *D. concentrica* were similarly treated.

7. Ascospore germination of *D. vernicosa* was markedly stimulated by short exposures to high temperatures previous to inoculation, and although the percentages of germination increased as the temperatures were lowered, even at 100° C. more germination

was obtained than in the controls. Similar treatment of ascospores of *D. concentrica* resulted in a decrease in germination. In both species it seems that short exposures at 90° C. have approximately the same effect as lower temperatures (70–75° C.).

8. Ascospores of *D. concentrica* on oatmeal, Leonian, Pfeffer, prune decoction, and *Platanus* decoction agars subjected to a temperature of 38° C. for 24 hours did not germinate at all or only poorly, but when changed to 25° C. recovered from partial plasmolysis or increased in percentage of germination. Pfeffer agar was the most favorable medium for germination.

9. The effect of these same media on ascospore germination of *D. concentrica* at room temperature was a maximum percentage on Pfeffer agar and a minimum on prune-decoction agar. For *D. vernicosa* and *Daldinia X* the maximum germination occurred on Pfeffer agar and the minimum on *Platanus* decoction agar.

10. In maltose solutions the maximum germination of one-year-old ascospores of *D. concentrica* was obtained in 10 per cent concentration, while the minimum was obtained in .06 per cent. The maximum germination of the month-old ascospores was obtained in 10 per cent solution, while the minimum was obtained in .05 per cent. The younger ascospores were more consistent in their behavior than the older ones. Obviously the ascospores of *D. vernicosa* were benefited by this rest of a year, for their germination far exceeded that of the month-old ascospores. The maximum germination of the former was 17.3 per cent, which occurred in a 3 per cent maltose solution, while that of the latter was 4 per cent germination secured at 10 per cent solution. Ascospores of *Daldinia X* 2 months old did not germinate in any of the concentrations.

11. After 48 hours on a medium of pH 5.6 ascospores of *D. concentrica* gave 70 per cent germination and a minimum of 23 per cent at pH 2.4; the optimum range was from pH 4.8 through 6.0. Conidia occurred at 10 different hydrogen-ion concentrations, while in *D. vernicosa* they occurred at only 5 different ones. The maximum germination, 17 per cent, occurred at pH 4.8, and the minimum, 4 per cent, at pH 2.4; the optimum occurred between pH 4.8 and 6.4.

12. For the effect of light and darkness on growth of mycelium,

oatmeal agar gave more abundant growth than Pfeffer agar. In both cases, darkness was more conducive to more rapid aerial and abundant growth than light. In the case of *D. concentrica*, only slight changes in color occurred, while in the light decided changes were noted. A red secretion appeared both in *D. vernicosa* and *Daldinia Y*, of which the mycelia closely resemble each other in pure culture.

13. In determining the effect of different temperatures and media on the growth of mycelium, Pfeffer agar proved to be the best medium for rapid growth of *D. concentrica*, *D. vernicosa* (D. Mo.) (C. Mass.), and *Daldinia Y*, but oatmeal agar induced much more abundant mycelium. Thirty-two degrees C. proved to be the most favorable temperature for *D. vernicosa* and *Daldinia Y*, while 23° C. proved to be most favorable for *D. concentrica*. More conidia were found on media rich in carbohydrates. Neither zoning nor the presence of conidia was dependent on differences of exposure to light.

14. As to the influence of different hydrogen-ion concentrations on the growth of mycelium of *D. concentrica*, a very narrow range of pH 6.2 and 6.6 produced the most abundant growth; the most rapid rate of growth, 3.5 mm. per day, occurred at pH 6.6. The most abundant growth of mycelium of *D. vernicosa* occurred in the range of pH 5.4, 5.8, and 6.2, but the fastest rate of growth, 7.11 mm., occurred at pH 5.0. This was the most rapid-growing mycelium of all three species. The range in which the most abundant growth of mycelium of *Daldinia X* was produced was pH 6.6 through 7.4, but the most rapid rate per day, 6.2 mm., occurred at pH 5.4. Although the ascocarp and ascospores of *Daldinia X* closely resemble *D. vernicosa*, in pure culture the behavior in regard to mycelium and conidia is very similar to that of *D. concentrica*.

15. These experiments definitely indicate that the physiological reactions of the fungi are correlated with their morphological characters.

Acknowledgements

In concluding, the writer wishes to express her indebtedness to Dr. David H. Linder for his stimulating enthusiasm, unstinted

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Explanation of Plate

PLATE 36

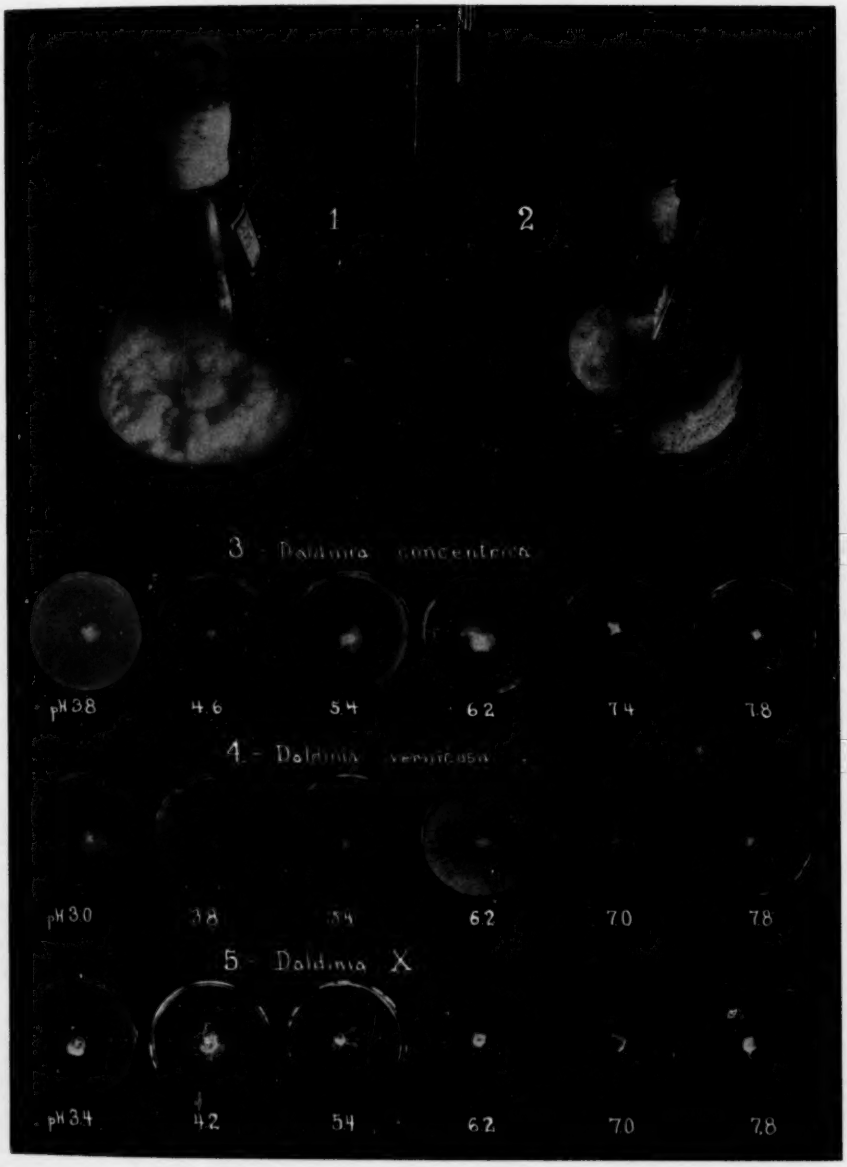
Fig. 1. The effect of *darkness* on the mycelial growth of *Daldinia Y* (B. S. D.) on oatmeal agar—after 30 days.

Fig. 2. The effect of *light* on the mycelial growth of *Daldinia Y* (B. S. D.) on oatmeal agar—after 30 days.

Fig. 3. The effect of different hydrogen-ion concentrations on the rate of growth of mycelium of *D. concentrica* (D. Mo.) 72 hours after inoculation. The numerous small blotches on the agar plates are not due to contamination but to undissolved bits of agar.)

Fig. 4. The effect of different hydrogen-ion concentrations on the rate of growth of mycelium of *D. vernicosa* (D. Mo.) 72 hours after inoculation.

Fig. 5. The effect of different hydrogen-ion concentrations on the rate of growth of mycelium of *Daldinia X* (V. P. Mo.) 72 hours after inoculation.



CHILD—DALDINIA

Explanation of Plate

PLATE 37

All figures were drawn with the aid of the camera-lucida, and were made from pure-culture material mounted in lacto-phenol cotton-blue.

Fig. 1. Ascospore of *D. vernicosa* (C. Mass.), showing the dark brown spore and the roughened nearly hyaline exospore. Drawn 24 hours after inoculation on bean-sprout agar. × 498.

Fig. 2. Ascospores of *D. vernicosa* (C. Mass.), showing the dark brown spore and the cracked mesospore. Drawn 24 hours after inoculation on bean-sprout agar. × 498.

Fig. 3. Germinated ascospores of *D. vernicosa* (D. Mo.). Drawn 24 hours after inoculation on Pfeffer agar. × 498.

Fig. 4. Germinated ascospore of *D. vernicosa* (C. Mass.), showing the flattened end of the germ-tube which became arrested in its development. Previous to inoculation the ascospore was frozen in sterile distilled water for 13 days at −5° C. Drawn 14 days after inoculation on Pfeffer agar. × 498.

Fig. 5. Germinated ascospore of *D. vernicosa* (D. Mo.). Drawn 48 hours after inoculation on Pfeffer agar. × 498.

Figs. 6 and 7. Germinated ascospores of *D. vernicosa* (D. Mo.). Drawn 24 hours after inoculation on Pfeffer agar with *Pleurococcus* alga and water in bottom of cell. × 498.

Fig. 8. Germinated ascospore of *D. vernicosa* (D. Mo.). Previous to inoculation the ascospore was heated at 80° C. for 10 minutes. Drawn 48 hours after inoculation on Pfeffer agar. × 498.

Fig. 9. Germinated ascospore of *D. vernicosa* (D. Mo.). Drawn 48 hours after inoculation on Pfeffer agar. × 498.

Figs. 10 and 11. Ascospore of *D. concentrica* (D. Mo.), showing the sloughing-off exospore and the longitudinal cleft in the light brown mesospore through which the germ-tube emerges. Drawn 24 hours after inoculation on bean-sprout agar. × 498.

Fig. 12. Ascospore of *D. concentrica* (D. Mo.), showing the sloughing-off exospore and the longitudinal cleft in the endospore through which the germ-tube emerges. Drawn 24 hours after inoculation on Pfeffer agar. × 498.

Fig. 13. Immature conidiophore and conidia of *D. vernicosa* (D. Mass.) developed from single ascospore on oatmeal agar. Note that the conidia arise as terminal swellings of the hyphae. Drawn 28 days after inoculation. × 498.

Fig. 14. Immature conidiophores and conidia of *D. concentrica* (D. Mo.) developed from single ascospore culture on oatmeal agar. Drawn 7 days after inoculation. × 498.

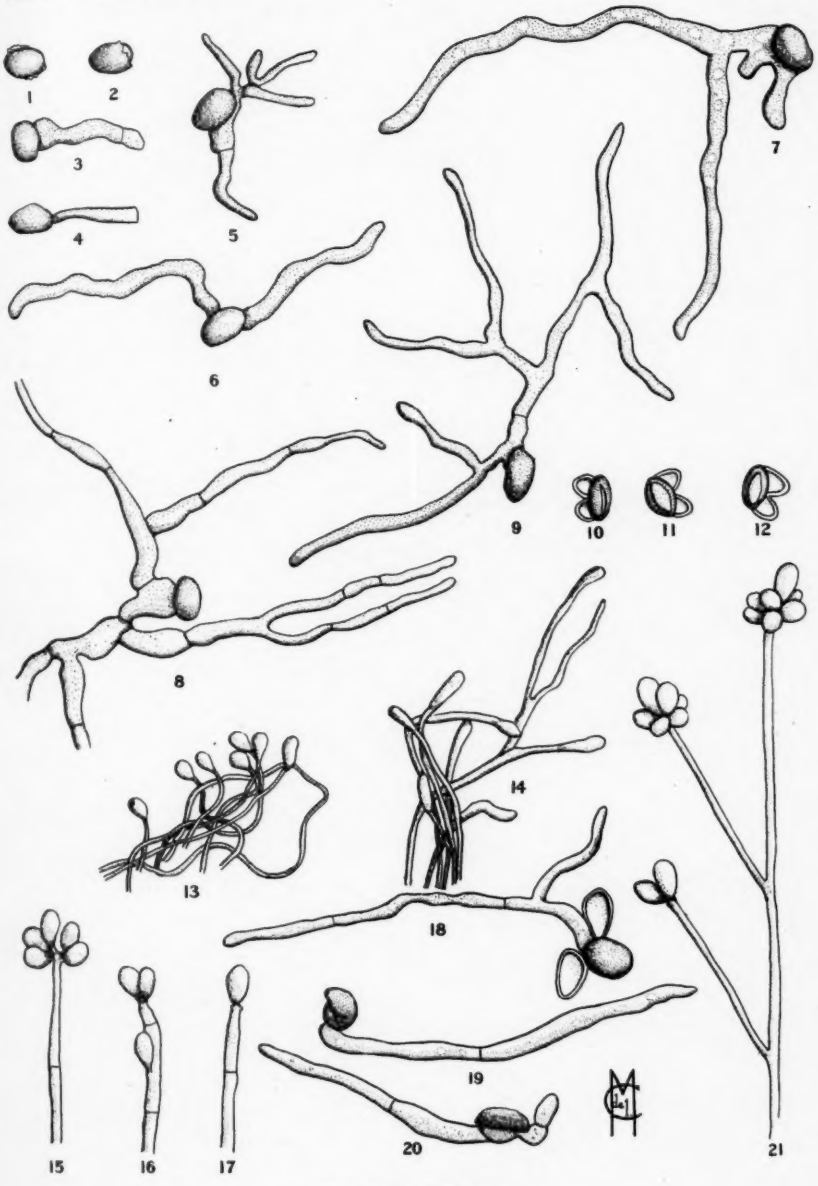
Figs. 15, 16, and 17. Mature conidiophores and conidia of *D. vernicosa* (C. Mass.) developed from single ascospore on oatmeal agar. Drawn 28 days after inoculation. × 498.

Fig. 18. Germinated ascospore of *D. concentrica* (D. Mo.). Drawn 24 hours after inoculation on Pfeffer agar with *Pleurococcus* alga and water in the bottom of the cell. × 498.

Fig. 19. Germinated ascospore of *D. concentrica* (D. Mo.). Previous to inoculation on bean-sprout agar the ascospore was heated to 100° C. for 2 hours. Drawn 10 days after inoculation. × 498.

Fig. 20. Germinated ascospore of *D. concentrica* (D. Mo.). Previous to inoculation on Pfeffer agar the ascospore was heated to 80° C. for 15 minutes. The characteristic splitting of the mesospore is shown. Drawn 48 hours after inoculation. × 498.

Fig. 21. Mature conidiophores and conidia of *D. concentrica* (D. Mo.) developed from single ascospore on Pfeffer agar. Drawn 17 days after inoculation. × 498.



CHILD—DALDINIA

Explanation of Plate

PLATE 38

Figs. 1 and 2. Germinated, very much swollen ascospores of *D. concentrica* (D. Mo.), showing the light brown mesospore and the hyaline endospore, developed on Pfeffer agar at pH 9.2. Drawn 19 days after inoculation. × 218.

Figs. 3 and 4. Germinated, very much swollen ascospores of *D. concentrica* (D. Mo.), showing the light brown mesospore and the hyaline endospore, developed on Pfeffer agar at pH 9.2. Drawn 19 days after inoculation. × 1071.

Figs. 5 and 6. Germinated ascospores of *D. vernicosa* (D. Mo.). Previous to inoculation on Pfeffer agar the ascospores were frozen at —5° C. for 38 days, then oxygen was run through the cultures at 6-hour intervals for 3 days. In fig. 6 the exospore, mesospore and endospore are evident. Drawn 120 hours after inoculation. × 218.

Figs. 7a and 7b. Swollen ascospores of *D. vernicosa* (D. Mo.) on Pfeffer agar at pH 2.6. Drawn 19 days after inoculation. × 1071.

Figs. 7c, 7d, and 7e. Mesospores cracked off. From ascospores on Pfeffer agar at pH 2.6. Drawn 19 days after inoculation. × 1071.

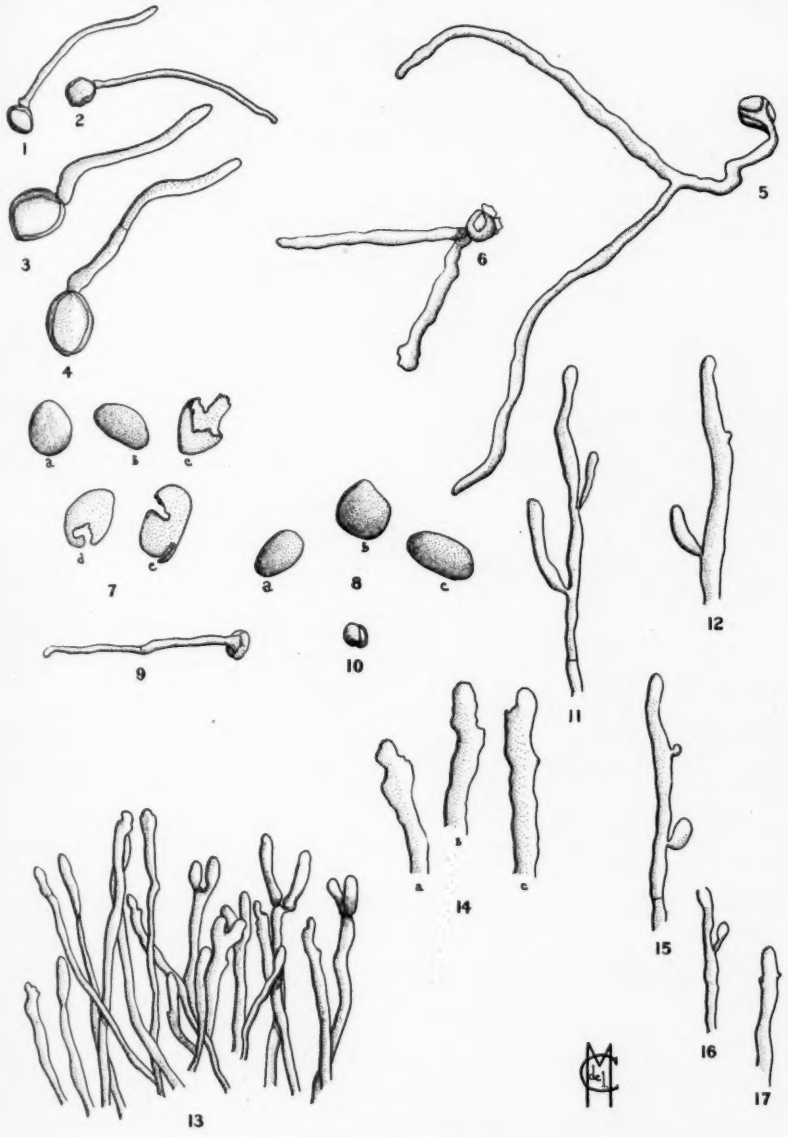
Figs. 8a, 8b, and 8c. Swollen ascospores of *Daldinia X* (V. P. Mo.). Previous to inoculation on Pfeffer agar, the ascospores were frozen at —5° C. for 3½ months. Drawn 24 hours after inoculation. × 1071.

Fig. 9. Germinated ascospores of *Daldinia X* (V. P. Mo.). Previous to inoculation on Pfeffer agar, the ascospore was frozen at —5° C. for 1½ months. Drawn 48 hours after inoculation. × 218.

Fig. 10. Ascospore of *Daldinia X* (V. P. Mo.), showing the sloughing-off exospore. Previous to inoculation on Pfeffer agar the ascospore was frozen at —5° C. for 1½ months. × 218.

Figs. 11, 12, 14a, b, c, and 15. Different stages of development of conidiophores and conidia of *Daldinia X* (V. P. Mo.), developed from a single ascospore on oatmeal agar. Drawn 120 hours after a bit of mycelium was transferred from the original culture to Leonian agar at pH 3.4. × 1071.

Figs. 13, 16, 17. Same as 11, 12, 14, and 15, but magnified × 218.



CHILD—DALDINIA

THE LIFE HISTORY AND CYTOLOGY OF SACCOBLASTIA INTERMEDIA, N. SP.

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The genus *Saccoblastia*, of the Auriculariales, was first described from Brazil by Möller ('95) from material that he had collected and studied. Subsequently several representatives of the genus have been described from Europe, and one from North Carolina in the United States. While its distribution is of interest, the real importance of the genus is emphasized by the amount of speculation as to the life history of its members, especially from the cytological point of view. For this reason, the writer has thought it well worth while to present his observations on an as-yet-undescribed species that he collected in Cuba.

The species of *Saccoblastia*, so far as can be determined from the literature, are all effused and fall into two groups,—those that are floccose or hypochnoid, and those that are of a gelatinous or mucous consistency. To this latter group, Bourdot and Galzin ('27) have applied the subgeneric name of *Saccogloea*, and it is to this subgenus that the species under discussion belongs. Both macro- and microscopically, however, it is distinct from all species that have been described up to the present time. For purposes of comparison, certain measurements of this and the related species have been tabulated. From the data thus presented, it is

Species	Probasidium	Basidium	Spores
S. sebacea	6–9 × 18–30 μ	4–6–9 × 45–75 μ	5–8 × 8–16 μ
S. sebacea var. *pruinosa*	7–9 × 12–18 μ	4.5–6 × 45–60 μ	4.5–7 × 6–10 μ
S. subardosiaca	7–9 × 30–36 μ	———	6.8 × 15–18 μ
S. caroliniana	8.5–16 × 24–45 μ	———	7–7.7 × 15–17 μ
Saccoblastia sp.	7.2–11 × 28–39.6 μ	5.4–9 × 64–72 μ	5.4–8.1–9 × 14–19.8–24 μ

clear that the Cuban material has a close resemblance to that from North Carolina, although differing in the size of the spores. A character not shown in the table, and one of great interest, is the presence of a second type of probasidium which is clavate and

resembles that of the genus *Iola*. This additional character, together with the fact that the colonies are not effuse but pustulate, makes it desirable to recognize this as a new species to which the name *Saccoblastia intermedia* is given because of the apparently transitional position between *Iola* and *Saccoblastia*.

Saccoblastia intermedia n. sp.

Fructifications pustulate, white to dilute brown, gelatinous to mucous, up to 1 cm. diam., becoming somewhat pendant at maturity. Context hyaline, the hyphae slender, 2.7–5.4 μ thick, without clamp connections. Probasidium of two types, the clavate type hyaline, rarely slightly constricted in the middle, 5.4–9 × (18)–36–45 μ, the walls slightly thickened; the lateral probasidium saccate, pendant, hyaline, 7.2–11 × 28–39.6 μ. Basidia hyaline, straight or slightly arcuate, 5.4–9 × 64.8–72 μ, 3–(5)-septate, bearing 4, less frequently as many as 6, sterigmata of varying length, 1.8–3.6 × 7.2–50 μ, the shorter ones conical. Basidiospores 1– rarely 2-celled, ellipsoid to elongate-ellipsoid, frequently concave on one side, tapering abruptly and obliquely to short truncate apiculi.

On moist decaying stump, Soledad, Cuba, September, 1924, *Linder* (TYPE in Farlow Herbarium, Herbarium of the Missouri Botanical Garden, and in the writer's herbarium).

Life History and Cytology

The material upon which the following account is based was preserved in alcohol of approximately 70 per cent strength. For study it was mounted in lactophenol to which had been added cotton blue and picric-nigrosin. The specimens as thus preserved in alcohol were plasmolyzed and the finer details of nuclear structure were lost. The lactophenol, however, practically restored the cytoplasmic structure, and as a result the nuclei in most of the material became clearly visible and were stained by the combination of cotton blue and picric-nigrosin.[1]

Examination of the pustules under the microscope reveals the fact that the fruiting body is not highly developed. The hyaline

[1] Weston, W. H. A useful modification of Amann's medium. Science N. S. 70: 455. 1929.

hyphae growing out radially in the gelatinous matrix soon give rise to peculiarly twisted or irregularly waved hyphae that connect two or more of the main hyphae (pl. 39, fig. 2, and pl. 41, fig. 1). The walls of these connectives are somewhat thicker than are those of the remainder of the vegetative hyphae, and are also slightly, though distinctly, colored brown. The peculiar growth indicates that the junction of the two hyphae is more than a mere anastomosis and that the wavy development is in reality the result of a stimulus that is sexual in nature. In other words, the connectives appear to take the place of clamp connections. Additional credence is given to this supposition by the abundance of such hyphae in the region below the first-formed probasidia. This is clearly shown in pl. 39, fig. 2, where the clavate probasidia are formed immediately above the junction of the hyphae with the connective.

The clavate probasidia are produced terminally on hyphae or on the ends of branches from them, by swelling. At the initiation of probasidium formation the protoplasm in the region involved becomes dense, and the nuclei are clearly seen in pairs for the first time. As enlargement of the tip of the hypha proceeds, the paired nuclei increase slightly in size and then migrate into the now clavate body (pl. 41, figs. 2–4) where they shortly fuse. The resulting nucleus is larger and, with its more deeply staining nucleolus, is clearly visible.

Just as in the clavate type of probasidium, the saccate type is also the seat of nuclear fusion, the only difference being that the paired nuclei migrate laterally into the sac-shaped outgrowth from the main hypha as is shown in pl. 40, figs. 1–3, and pl. 41, figs. 5–7. The saccate probasidia, although produced concurrently with the clavate type, do not appear until later in the life history of the fungus. If, as has been assumed earlier in this paper, *Saccoblastia intermedia* is transitional between *Iola* and *Saccoblastia*, then it seems possible to assume the saccate probasidia to be a secondary type that allows proliferation, and is the type that has become fixed in the remaining species of *Saccoblastia*. Not infrequently, the growing point of the saccate probasidium, the base in the earlier stages of development, continues its growth by producing a germ-tube (pl. 40, fig. 4, and pl. 41, fig. 9)

in which case it appears to be transitional between the two types of probasidia. Also, although more rarely, the saccate type may germinate at the basal and apical end (pl. 41, fig. 10). Usually, however, such exceptions are infrequent, and the normal method of germination is by apical growth (pl. 41, fig. 8). At all events, the paired nuclei migrate laterally into the small immature probasidium and very quickly fuse. The rapidity with which this migration and fusion take place becomes evident when the sizes of the sacs, shown in pl. 41, figs. 5–7, are compared.

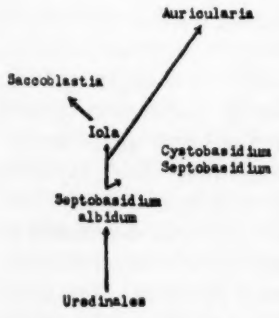
After a short rest, the probasidium sends forth a long hypha, or promycelium, which eventually enlarges terminally to become the true basidium. The fusion nucleus remains behind in the probasidium until the hypha attains full length, then there is a rapid migration of the nucleus into the basidium, followed by the entire protoplasmic contents of the probasidium and hypha (pl. 41, fig. 1). That the migration of the nucleus is rapid can only be inferred from the fact that the nucleus has never been observed by the writer anywhere but in the probasidium or the true basidium, never in the hypha connecting the two. Once the basidium is formed, it is most frequently 3-septate and each of the resulting cells has but a single nucleus. The nucleus and the protoplasmic content of each cell eventually migrate into the spore as it is formed so that the probasidium after it has produced the spores is nothing but an empty shell (pl. 39, fig. 1, and pl. 41, figs. 13 and 17).

Discussion

From the above account of the life history of this species it is obvious that, with the fusion of the paired nuclei taking place in the probasidium, that organ is actually homologous with the teleutospore of the rusts. Furthermore, the manner of germination of the basidium, that is, spore production, is strikingly like that found in the rusts. The resemblance between the probasidium and the teleutospore has been remarked upon since the genus *Saccoblastia* was first described. Möller ('95) in his account of the genus says: "Auch unter den Uredinaceen giebt es ja Formen, bei denen die Teleutospore ohne längere Ruhepause unmittelbar zur Basidie auskeimt. Durch diese Formen wird die nahe Ver-

wandtschaft unserer Saccoblastia-Arten mit den Uredinaceen besonders deutlich." Coker ('20) also mentions this relationship in the following extract from his account: "This genus reminds one strongly of the rusts, particularly the genus Gymnosporangium, where the teleutospore sprouts as soon as formed. If the relationship is as close as it seems the pear-shaped sac would be the homologue of the teleutospore. Note that this sac when long is constricted in the middle." While other writers point out this affinity, Coker is the only one who implies that the genus is derived from the rusts. Thus, Möller ('95), Neuhoff ('24), and Gäumann ('26), in their phylogenetic trees, indicate that this genus and the family to which it belongs, the Auriculariaceae, arose before the rusts. The main objection to Möller's scheme of evolution is based on the fact that in the Ustilaginaceae the nuclei in the cells of the basidium divide into two daughter nuclei, one of which migrates into the spore, the other remaining in the basidial cell. In *Saccoblastia* such is not the case, for the nucleus migrates into the spores with the protoplasm of the cell after the manner of *Gymnosporangium* or other members of the Pucciniaceae. To Neuhoff's and Gäumann's system it may be objected that a form with a relatively simple life-cycle should not be selected as the source of forms with a very complicated one.

From the nature of the subject, discussions of phylogeny are highly theoretical, yet it seems to the writer that the evidence pointing to the derivation of the Auriculariales from the Uredinales is stronger than the reverse. The evolutionary tendency seems to be from the rusts to *Auricularia* somewhat in the manner shown briefly in the accompanying text-figure. It is quite possible that the group as a whole is polyphyletic in its origin, but in this scheme the chief idea is to show trends in certain characteristics. Thus we find among the Pucciniaceae a tendency to drop out one or more steps in the life-cycle. Also, as is true in *Gymnosporangium*, there is a marked shortening of the



rest period of the teleutospore. If now it can be supposed that during the past history of the family, either through gradual evolution or through mutation, a race arose that was weakly parasitic or even saprophytic, then we arrive at the forerunner of a genus similar to *Septobasidium* or *Cystobasidium* in which the thickened walls of the probasidium still are in evidence. In this connection, it is unfortunate that Patouillard ('13) was unable to make a cytological study of the toruloid spores of *Septobasidium albidum*. Had careful investigation shown the spores to be binucleate, then there would have been demonstrated an even closer relation between the rusts and *Septobasidium* since in that case the toruloid spores might with reason be considered homologous with aeciospores.

The next step in advance would be a genus similar to *Iola* of which the probasidium has practically lost its thickened walls. Through *Saccoblastia intermedia* with its clavate and saccate probasidia there appears to be a natural transition from *Iola* to *Saccoblastia*. The fact that the clavate probasidia are the first produced, and that they are found only in the older material, is significant. Since the clavate type is terminal, it is apparent that in order to produce additional probasidia with the least waste of material the subsequent probasidia must be produced laterally from the binucleate hyphae. This is what appears to have happened, but the remaining members of the genus *Saccoblastia* have retained the saccate type to the exclusion of the clavate.

Reduction of the probasidium is continued until in *Auricularia* we find, according to Gäumann and Dodge ('28, page 542), the place of fusion of the nuclei indicated only by a slight swelling. The branch of the family tree terminated by *Auricularia* appears to have arisen slightly before *Iola* since the latter genus apparently has lost the asexual method of reproduction. In *A. auricula-judae* the paraphyses have been shown by Sappin-Trouffy ('96) to be binucleate. These binucleate cells might be considered as homologous with the conidia produced by *Septobasidium albidum*, although they have lost their reproductive function. The binucleate condition of the spores of *S. albidum* would not be unparalleled, for Dangeard ('95) has shown the presence of binucleate oidia in *Dacryomyces deliquescens* of the Dacryomycetales. Should

the conidia of *S. albidum* or other species of *Septobasidium* eventually prove to be binucleate, then there would be slight reason for doubting the homology between such conidia and aecidiospores.

Throughout this theoretical discussion, it will be observed that the present hypothesis is founded on the reduction of the teleutospore or probasidium, on the reduction of the aeciospores, on the development of an extensive fruiting body, and finally on the transition from the parasitic mode of existence to the saprophytic. Still another phase of the subject that has been considered, though not discussed for lack of available knowledge, is the relation of the sporidia to pycnidiospores. Brefeld ('88), however, has remarked on the resemblance between the two, and has called attention to the fact that he was unable to obtain germination of the sporidia.

It is not necessary to consider the sporidia homologous with the pycnidiospores, although a study of the cytology and the relationship of these spore forms would undoubtedly bring to light much very interesting information. To the writer's way of thinking, it is sufficient to realize that the morphology of the basidiospores of the rusts and Auriculariales is fairly constant throughout the groups. Therefore, to find that the spores of certain rusts, such as *Cronartium ribicola*, also produce sporidia is sufficient evidence to show that there is a very close relationship between the two orders.

All factors considered, and in the light of present-day knowledge, it would appear that the Auriculariaceae are derived from the Uredinales through the reduction or degeneration of the reproductive organs as a result of the saprophytic mode of existence. To have the Uredinales derived from the Auriculariales would presuppose the development of potentialities in organs or even the development of the organs themselves. The probasidia of the Auriculariales have all the evidence of being vestigial structures handed down from the Uredinales, even though the latter order be derived from the Red Algae, the Zygomycetes, or even, as seems most probable, from a parasitic group of Ascomycetes.

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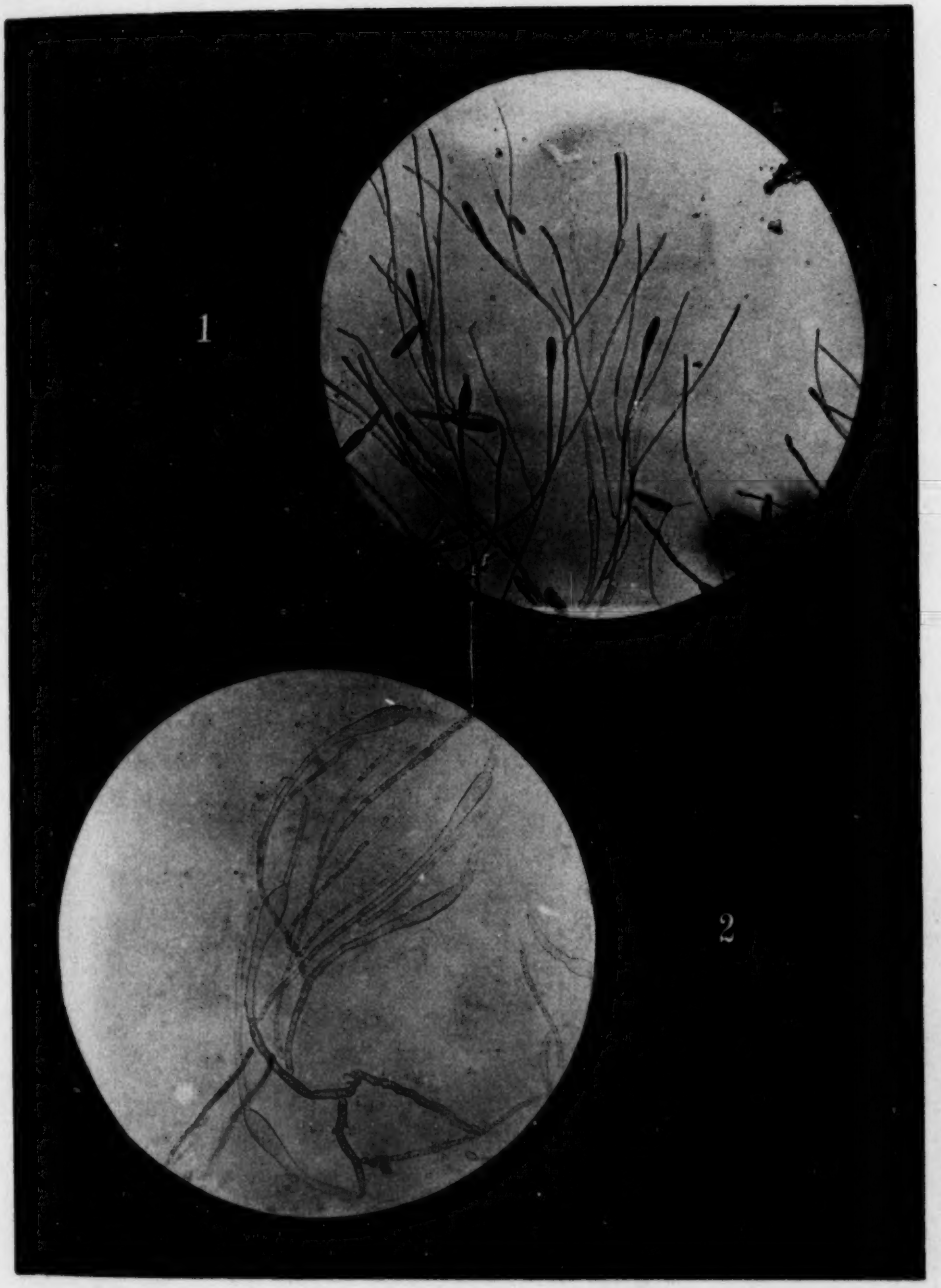
Explanation of Plate

PLATE 39

The material from which all photographs and drawings were made was mounted in Amann's lactophenol to which had been added cotton blue and picric-nigrosin. The photographs were made on Eastman panchromatic plates with the aid of a Wratten "A" (red) filter.

Fig. 1. Photograph at a low magnification to show the characteristic features of *Saccoblastia intermedia.* In the middle of the picture, near the bottom, will be seen an empty probasidium which has given rise to a true basidium, barely outlined above. The latter has been emptied of its contents through the production of basidiospores. An immature saccate probasidium with its fusion nucleus is shown to the left of the empty basidium, while immediately below the point of attachment of the probasidium, on the same hypha, are paired nuclei that have not fused. Below these are two nuclei that are approaching the paired condition. Approx. × 120.

Fig. 2. Clavate probasidia are shown arising after the fusion of the connective hypha with the main one. Paired nuclei may be observed in the hyphae between the two uppermost probasidia. Approx. × 240.



LINDER—SACCOBLASTIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 40

The magnification in all instances is approximately × 700.

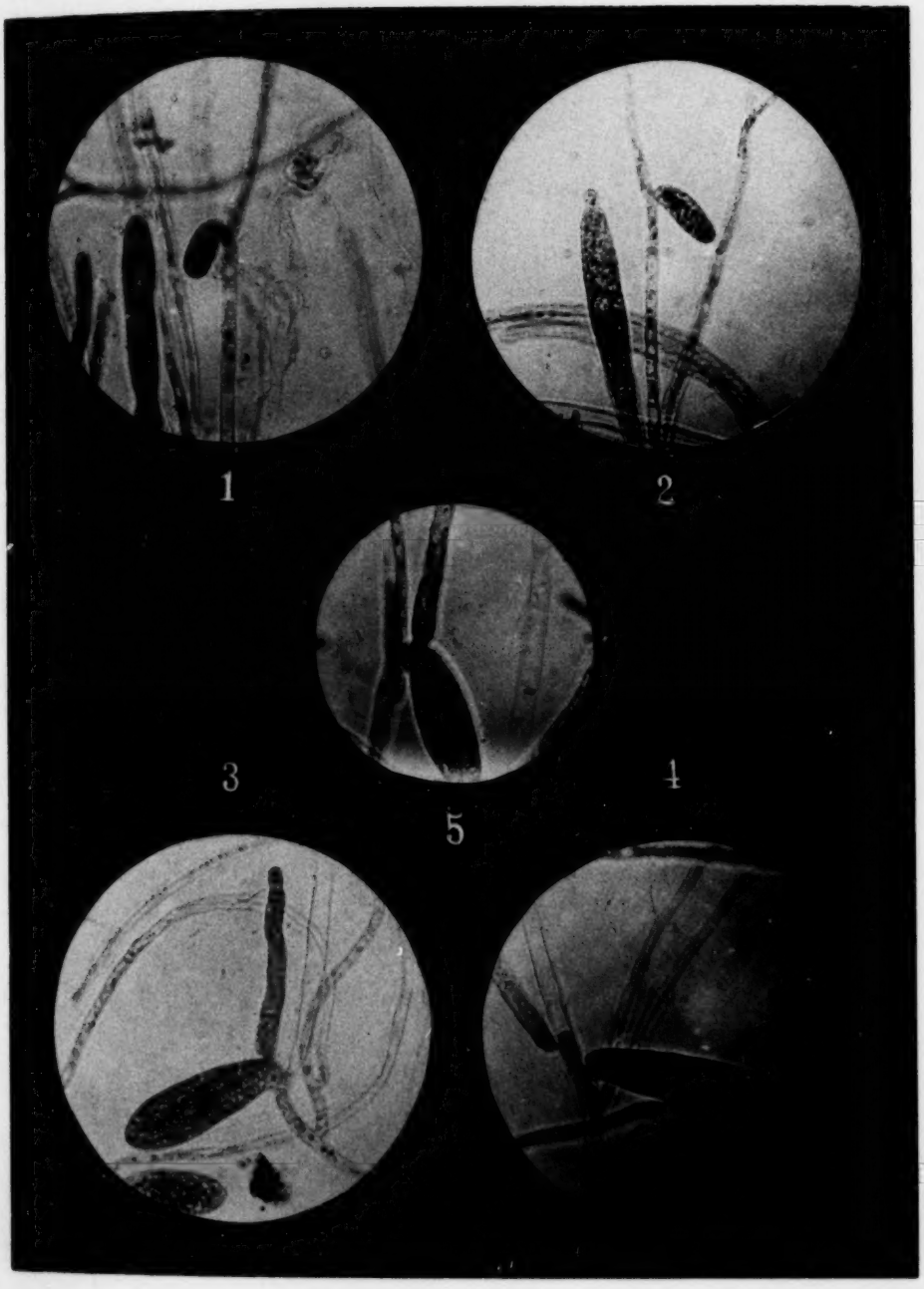
Fig. 1. A young probasidium into which one of the paired nuclei has migrated. The other nucleus is about to enter. Below and on the same hypha are two nuclei which have associated in pairs. See also pl. 41, fig. 5.

Fig. 2. A young probasidium in which the two nuclei have become closely associated and are about to fuse. See also pl. 41, fig. 6.

Fig. 3. A germinating probasidium with its conspicuous fusion nucleus.

Fig. 4. A probasidium continuing apical growth by the production of a promycelium from what is ordinarily the basal end.

Fig. 5. A germinating probasidium showing the conspicuous nucleus with its more deeply staining nucleolus. The promycelium is more than four times as long as this particular probasidium.



LINDER—SACCOBLASTIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 41

All drawings have been made with the aid of a camera lucida and are shown at a magnification of × 600.

Fig. 1. Shows the peculiarly wavy and somewhat twisted connective hypha that is formed prior to the production of probasidia. Such hyphae appear to take the place of clamp connections. See pl. 39, fig. 2.

Figs. 2–4. Formation of the clavate type of probasidium.

Figs. 5–8. Formation of the saccate type of probasidium. Figs. 5 and 6 are drawn from the same probasidia as are shown in pl. 40, figs. 1–2.

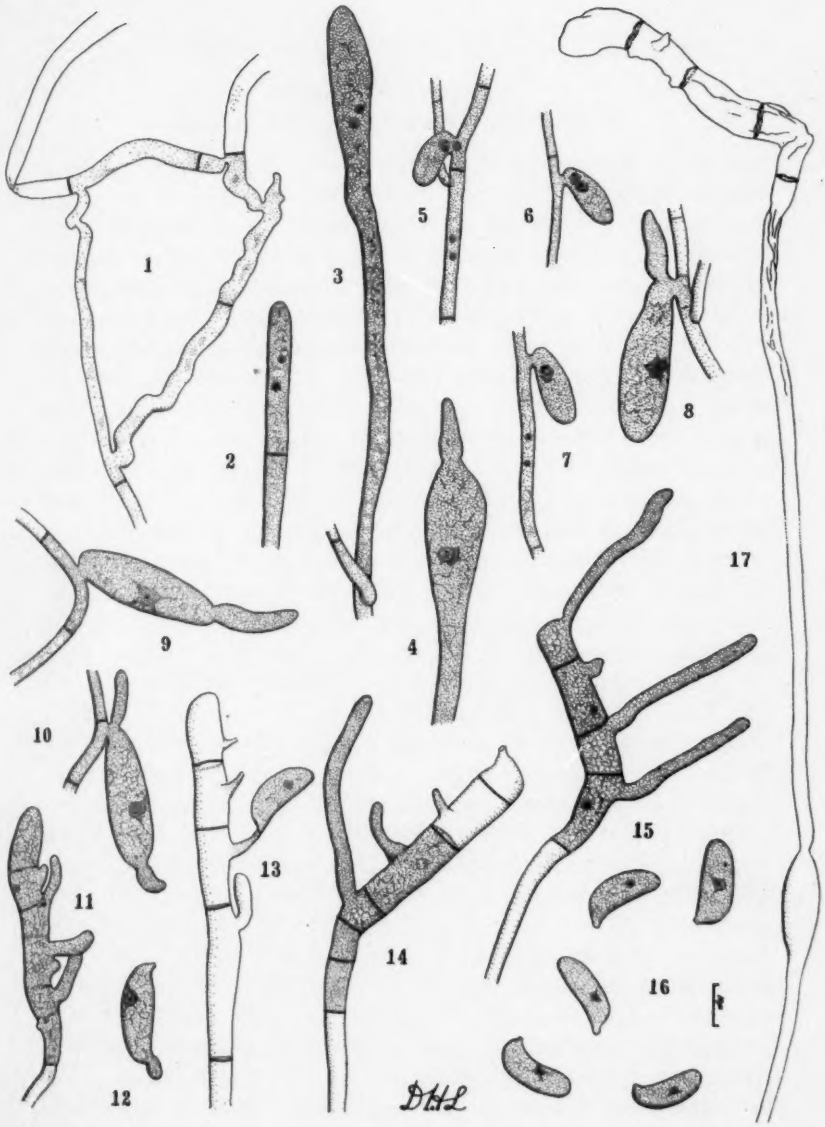
Figs. 9–10. The abnormal germination of probasidia. In fig. 9 the promycelium is arising from the basal end of the probasidium, while in fig. 10 each end is giving rise to a promycelium.

Figs. 11, 13–15. Types of true basidia. In fig. 13 spores have been produced but only one remains attached. It will be noted that not all of the protoplasm of the basidial cell has been able to enter the spore. In the remaining basidia are shown variations in the size of the sterigmata and number of septations of the basidia.

Fig. 12. A germinating basidiospore.

Fig. 16. Basidiospores, all of which contain but one nucleus apiece.

Fig. 17. A probasidium which has given rise to a promycelium and basidium. The promycelium is somewhat shortened After the basidium has produced the basidiospores, it collapses and finally disintegrates. Compare with that shown in pl. 39, fig. 1.



LINDER—SACCOBLASTIA

COCKAYNE, BOSTON

NON-SYMBIOTIC GERMINATION OF ORCHIDS

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The propagation of orchids from seeds is a problem which has attracted the attention of many investigators. That orchids could be grown in the greenhouse from seeds was not considered extraordinary; orchid growers in Europe and in this country were successful in this line of work. Naturally scientists took up the study of the conditions of germination. In the roots of the orchid plants symbiotic fungi had been detected, and the first investigators of this problem, Bernard ('09) and Burgeff ('09) undertook experiments with the symbiotic fungus. Since they had no success without the fungus, the presence of the fungus was considered to be a *conditio sine qua non*. In 1922 Knudson published the results of his successful experiments on the non-symbiotic germination of orchids, and a number of other investigators have worked on the same subject, in the majority of cases successfully. It is a well-known fact that a greater knowledge of the conditions for growing orchids is of particular interest not only to those working on the subject for commercial purposes, but also to those engaged in scientific investigations.

From Knudson's ('25, '27) experiments we learn that the metabolic processes of the fungus bring about chemical changes in the medium which make it more assimilable. Chemical compounds indigestible for the seedlings are broken up or transformed into ones which can be easily used as food by the embryo. Another favorable influence of the fungus is the gradual acidification of the medium, according to Burgeff, Knudson, and Clement. However, experiments showed that it need not be necessarily the special orchid fungus. Other fungi may produce the same effect. Every one dealing with the germination of orchid seeds will have made the same discovery. From the experiments of Knudson and other investigators, such as Constantin and Magrou ('22), Ramsbottom ('22), and Clement ('24, '26, '29), our present knowledge of the requirements for successful germination of

orchid seeds can be summed up as follows: a suitable solution of mineral salts, a satisfactory sugar supply, and a favorable hydrogen-ion concentration. These are conditions which can be obtained by a nutrient solution, the composition of which has been determined by previous investigations. This has been proved in a great number of experiments on seeds from different species of orchids, and there is no doubt that further investigations will show that the theory holds for all species of orchids. If we take into consideration these three main requirements, mentioned above, and determine, by changing the composition of the medium, the best conditions of germination, we will be able to give a list of the most favorable nutrient solutions for germinating seeds of each species of orchids.

In preliminary experiments nutrient solutions containing only sugars, three-times distilled water, and agar (Merck's Reagent) which had been washed for three weeks in thrice-distilled water and carefully dried in a desiccator over concentrated sulphuric acid, were used. Maltose, glucose, levulose, and saccharose (Merck Reagents) were used as sugar supplies. The best germination and growth of Cattleya seeds were recorded on nutrient media with maltose and at pH values between 4.8 and 5.4. The seedlings developed two leaves within five months and appeared healthy. Transplanted to full nutrient solutions with a suitable sugar supply development and growth continued as in seedlings germinated the usual way. The results prove the importance of carbohydrates in the germination of orchid seeds. For this reason the experiments with solid media were carried out and the results are reported here.

It is hoped that the experiments reported in this paper may be a contribution to the subject.

METHODS

As culture vessels Erlenmeyer-flasks of "Pyrex" glass of 250-cc. capacity were used. One hundred cc. of nutrient agar was poured into each flask, the neck of the flasks closed with a cotton plug, and the flasks sterilized in an autoclave for 30 minutes under a pressure of 10 lbs. Then the medium was allowed to cool in a slanting position. The medium was so

adjusted before sterilization that when the process was concluded the desired pH resulted, as was shown by tests. All seeds were sterilized in chlorinated lime solution for 30 minutes, according to the procedure recommended by Wilson ('15). During this time the solution was shaken frequently to moisten all seeds. The sterilizing solution was then poured out and sterilized distilled water added under sterile conditions, and in this the seeds remained until they were planted on the agar slant with the aid of a sterilized platinum loop. After the seeds were planted, the cotton plug and the neck of the culture flask were covered with a double layer of waxed paper fastened with a rubber band to prevent too rapid an evaporation of the water, especially in extremely hot weather. Conditions in the greenhouse made it necessary to add sterile water to the cultures from time to time, usually after each sixth week. Although this was done with all possible precautions contamination, which often destroyed as many as 10 per cent of the experiments, could not be avoided. Therefore all experiments were carried out in triplicate.

It might be mentioned here that a number of different nutrient solutions was tried out with regard to the effect on germination of orchid seeds. The nutrient solutions used were those employed by Artari, Molisch, von der Crone, Nobbe, Pfeffer, Sachs, and three different variations of Knop's solution. Although a definite decision cannot be made at present, the best growth and development were recorded in the nutrient solutions employed by Knop and Sachs. However, the growth of seedlings in these solutions was only 50 per cent of that obtained during the same period in the solutions of Burgeff, Pfeffer, and Knudson's solution "D." Since the nutrient solution "B" employed by Knudson ('22) and the one developed by the writer, here called solution "L," proved most satisfactory, they alone are considered in this paper.

Solution B		Solution L	
$Ca(NO_3)_2$	1.00 gm.	$CaCl_2$	1.00 gm.
K_2HPO_4	0.25 gm.	KH_2PO_4	1.00 gm.
$MgSO_4 . 7H_2O$	0.25 gm.	$MgSO_4 . 7H_2O$	1.00 gm.
$Fe_3(PO_4)_2 . 4H_2O$	0.25 gm.	$Fe_3(PO_4)_2 . 8H_2O$	0.33 gm.
$(NH_4)_2 . SO_4$	0.50 gm.	$(NH_4)_2 . CO_3 . H_2O$	0.50 gm.
Sugar	20.00 gm.	$NH_4 . NO_3$	0.50 gm.
Distilled water	1000 cc.	Sugar	20.00 gm.
		Distilled water	1000 cc.

In general 1.75 per cent agar was added. Preliminary experiments had shown that orchid seeds planted on medium L finally grew as well as in Burgeff's solution. Acid was added in a quantity necessary to bring the pH concentration to the point desired. Of course, in solutions with pH values below 4.8 the percentage of the agar had to be raised in order to render the medium solid enough for the purpose designated. After planting, the culture flasks were placed in the greenhouse at a temperature between 20° and 30° C. and kept covered with cheese-cloth.

It was possible to make measurements with an ocular micrometer attached to the microscope, but only of those seedlings growing close to the wall of the flask. However, since the number of seedlings in one flask never exceeded 300, the seedlings in the same stage of growth and of the same size could be examined with relative ease and the percentage of the different sizes determined.

The seeds used in these experiments were those from *Cattleya* hybrids grown at the Missouri Botanical Garden, and were over one year old. The parents are as follows:

No. 18. *Cattleya Trianae* × *Laelio-Cattleya luminosa aurea.*

No. 21. *Cattleya Trianae* × *Cattleya "Princess Royal" alba.*

No. 27. *Cattleya Trianae* (*light*) × *Cattleya O'Brieniana.*

In general, the terms used in describing the stages of development are those employed by Knudson ('22); the expression "advanced spherule stage" used in this paper means the period of production of leaves before the formation of the first root.

Experiments

Experiments were carried on with solution B and the writer's solution L, to which had been added levulose, glucose, sucrose, or maltose, as the source of sugar. The seeds were planted on July 19, 1927, and the results here reported recorded on December 29, 1927.

The measurements were taken in the manner described above; for this reason the figures given are only approximate, but are under- rather than over-estimated. The numbers given in the column "succession in superiority" express the following three stages, the average measurements of the embryos being used: (1) average diameter above 1000 μ, (2) average diameter from 500 μ to 1000 μ, and (3) average diameter below 500 μ.

The results of these experiments, recorded in table I, prove that the best growth was obtained in nutrient solutions with maltose as the sugar supply.

TABLE I

CATTLEYA SEEDS PLANTED ON JULY 19, 1927; GROWTH RECORDED ON DECEMBER 29, 1927

No. of seed	Nutrient solution	Diameter of embryo in microns			State of growth	Succession in superiority
		Min.	Max.	Aver.		
18	Plain B	90	140	115	Small spherule stage; very light green; seedlings apparently dying.	3*
21		85	180	123		3
27		100	200	150		3
18	Solution B + 2% levulose	460	1200	830	Seedlings forming leaves; green; healthy.	2
21		490	1500	995		2
27		500	1400	950		2
18	Solution B + 2% glucose	460	1100	785	Seedlings green; forming leaves; well developed.	2
21		480	1200	840		2
27		450	1300	875		2
18	Solution B + 2% sucrose	450	1000	725	Seedlings well developed; green; forming leaves.	2
21		460	900	680		2
27		500	1100	800		2
18	Solution B + 2% maltose	850	1000	925	Seedlings very well developed; green; slender; healthy; forming leaves.	2
21		960	1100	1030		1
27		1000	1200	1100		1
18	Plain L	100	180	140	Small spherule stage; very light green; leaf point marked.	3
21		90	200	145		3
27		100	300	200		3
18	Solution L + 2% levulose	520	1350	935	Seedlings well developed; green; healthy; forming leaves.	2
21		680	1400	1040		1
27		700	1500	1100		1
18	Solution L + 2% glucose	550	1150	850	Seedings well developed; green; forming leaves.	2
21		670	1350	1010		1
27		540	1450	995		2
18	Solution L + 2% sucrose	490	1200	845	Seedlings well developed; green; forming leaves.	2
21		630	1400	1015		1
27		480	1500	990		2
18	Solution L + 2% maltose	890	1400	1145	Seedlings very well developed; dark green; very well developed protocorms; all forming leaves.	1
21		860	1500	1180		1
27		1000	2000	1500		1

* The numbers in this column denote the following stages: 1, average diameter above 1000 microns; 2, average diameter from 500 to 1000 microns; and 3, average diameter below 500 microns.

SOLUTION B

Maltose.—The best growth was recorded in solutions which had been supplied with maltose. Ninety per cent of the embryos were far advanced in growth, having protocorms from 1 to 1.2 mm. in diameter; each had developed two leaves (3 to 6 mm. in length). Ten per cent of the seedlings had already produced one leaf (2 to 4 mm. in length), and a second one half as long as the first was forming. All seedlings had a great number of fine root hairs and appeared healthy. The internodes and petioles were elongated and showed only a very pale green color, this condition resembling the etiolation usually ascribed to lack of nitrogen. However, development of the seedlings was otherwise apparently normal, and the leaves were vigorous, as shown by their large size. The etiolated cases in which growth was retarded may be considered as individual variations (seedlings which because of some internal factors grew more slowly). The pH concentration in these cultures was between 5.2 and 5.4, certainly sufficient to secure normal growth, as preliminary experiments had shown. Seedlings grown on media with a pH concentration above 5.6 showed very slow growth and chlorosis. They were, at the time when the experiments were recorded, in the advanced spherule stage, but were only very light yellowish with a light green spot marking the place of the leaf point. In nutrient solutions with a pH concentration above 6.0 the growth was as poor as in the plain solution without any sugar supply.

Sucrose.—All seedlings were in the advanced spherule stage, about 60 per cent of them having already formed the first leaf (0.5 to 0.8 mm. in length) and the second one just started. The rest of the seedlings were forming the first leaf. All were healthy, but the color of the leaves was not as dark as in those seedlings grown on the maltose medium. The pH concentration of these solutions with sucrose was 5.2. (On the more alkaline media the growth and development of the embryos rapidly decreased, so that at a concentration of 5.6 the seedlings showed severe chlorosis). All were in the advanced spherule stage, but only 9 per cent had formed one leaf measuring 0.3 to 0.5 mm. in length, and the remaining 91 per cent had just reached the advanced spherule stage with the leaf point barely marked. All the embryos were

very light green, except those grown at 5.2, which were almost white, only the leaf point, or in the advanced stage of growth, the leaves, showing a very light green color. Two to three weeks after these experiments were recorded even this very light green color had almost faded away, and the seedlings were apparently dying, being unable to produce chlorophyll and to carry on assimilation.

Glucose.—The development of seedlings on this medium was fairly good. They had well-developed light green protocorms; 22 per cent had already produced two leaves (1 to 1.5 mm. in length); 78 per cent were just forming the first two leaves (0.5 to 1.2 mm. in length), the second leaf one-half the length of the first. All leaves were light green, and all seedlings which had attained full growth of both leaves had formed root-hairs. The pH concentration of the nutrient medium was 5.0. In experiments with higher pH values, likewise in the experiments with maltose and sucrose, a decrease in growth and life processes has been observed. At a pH concentration above 5.6 the development was noticeably retarded; the first indication of chlorosis appeared; the embryos reached the spherule stage and even pointed the first leaf, but were not able to continue in growth. In pH concentrations of about 5.8 and higher the seedlings swelled, but the embryos remained small and showed only very light yellowish green color.

Levulose.—The development of the seedlings on this medium was very satisfactory. The seedlings were dark green, the protocorms very well developed; about 10 per cent had already formed two dark green leaves (2 to 3 mm. in length); 65 per cent had well-developed protocorms and two dark green leaves, the second one-half the length of the first. Twenty-five per cent had not progressed as far in growth and were just forming the first leaf. The effect of the pH concentration was observed to be the same as in the media mentioned above. Of the remaining 25 per cent almost all were forming two dark leaves, the protocorms being green and well developed. Only very few had not progressed as far in growth and were just forming the first leaf.

In one of the cultures made from seed No. 27 about 38 per cent of the seedlings had died in the very first spherule stage; they had

swelled but turned brown before the leaf had been pointed; 7 per cent had passed the advanced spherule stage, had green protocorms and two dark leaves (2 to 3 mm. in length), and appeared very healthy. The failure of those 38 per cent to germinate could be explained only by the weak constitution of the seed, the nutrient medium having been the same as in the other nutrient solutions. This is mentioned merely to make a complete report on the experiments.

Plain nutrient medium.—On this medium without any sugar supply the embryos grew to the small spherule stage, the protocorms being about 90 to 150 μ in diameter and showing a whitish green color with a very light green spot, the leaf point. Apparently they were dying.

SOLUTION L

Maltose.—As in solution B, in solution L, the best growth of the seedlings was registered with maltose. The embryos were very well developed, with large and dark green protocorms (1 to 2 mm. in length) and dark green leaves. Eighty per cent of the seedlings had already formed the first leaf (2 to 3 mm. in length), the second one being half the length of the first. Ten per cent had passed through the small spherule stage and the first leaf was forming. Ten per cent had two leaves (2 to 5 mm. in length) and were forming the third one. The seedlings on this nutrient medium had the largest protocorms of any in these experiments; the protocorms were green, the leaves dark green, and the seedlings with developed leaves had formed root-hairs. All seedlings appeared normal, there being no unusual elongation of the internodes or the petioles, even though they were very crowded. It seems to be necessary to mention this fact, because one might suspect that in solution B with maltose the crowded condition of the seedlings might have caused the kind of etiolation mentioned above. The seedlings were in the best state of growth and health. The pH concentration of this nutrient medium was about 5.2. Another fact which ought to be brought out is that some of the seedlings with developed leaves showed the first signs of root formation.

Sucrose.—The seedlings on this medium were well developed with green protocorms and leaves somewhat darker, but not as

dark as in the cultures with maltose. Eighty per cent of the embryos had already formed one leaf (0.5 to 1 mm. in length) and were forming the second one about one-third the length of the fully developed leaf. Fifteen per cent were forming the first leaf (half of the full length), and 5 per cent were in the advanced spherule stage, just forming the first leaf. All seedlings appeared healthy, and those with developed leaves had formed root-hairs. The pH concentration was about 5.2.

Glucose.—The seedlings grown on glucose medium were fairly well developed and green with a well-developed protocorm. Sixty-eight per cent had two leaves (1 to 1.5 mm. in length); 32 per cent were forming the first leaf which was about 0.3 to 0.8 mm. in length. The embryos with developed leaves were forming root-hairs. The seedlings on glucose medium developed somewhat differently from those grown on a maltose or levulose medium. They grew more slowly and were light green, the leaves or the leaf points being dark green. The pH concentration was 5.2. On media with a pH value of 5.6 and above, decided chlorosis was noticed, and at a pH value of about 6.0 the growth equalled that on plain nutrient medium without any sugar supply.

Levulose.—Twelve per cent of the seedlings were dark green. The protocorms were very well developed, and compared to those on the other media were second largest in diameter. They had two developed leaves (1.5 to 2 mm. in length) and were forming root-hairs. Seventy per cent of the seedlings were dark green with one developed leaf (1 to 1.5 mm.), and 18 per cent were in the advanced spherule stage, just beginning to form the first leaf. Although the protocorms and leaves were well developed the growth was apparently slower than on the other media at the same pH concentration. The most favorable hydrogen-ion concentration was about 5.2.

Plain nutrient medium.—On this medium without any sugar supply the seedlings had reached the small spherule stage, the protocorms measuring about 90 to 200 μ in diameter. They had attained the same stage of growth in 5 months as those grown on sugar media in 4 to 6 weeks. The protocorms were very light green and showed a darker spot, the leaf point, and some of the seedlings had started to form a leaf (20 to 50 μ in length). Ap-

parently, however, they were not capable of carrying on assimilation. One month after these records were taken (January 29, 1928) the seedlings were still alive.

Discussion

Summarizing these results in a synoptical table we have the following data regarding the development on the different media.

TABLE II

	Solution B	Solution L
Plain	Very weak growth; seedlings apparently dying.	Weak growth; leaf-point weakly marked; continuation of growth doubtful.
+ levulose	10% with two developed leaves; 65% with one developed leaf, forming the second one; root hairs; 25% forming the first leaf. All apparently healthy.	12% with two developed leaves and root-hairs; 70% with one developed leaf; 18% in the advanced spherule stage, forming the first leaf.
+ glucose	22% with two developed leaves and root-hairs; 78% with one leaf, forming the second. Healthy.	68% with two developed leaves and root-hairs; 32% forming the first leaf. Healthy.
+ sucrose	60% with one developed leaf, the second forming; 40% forming the first leaf.	80% with one developed leaf, the second forming; root-hairs; 15% forming the first leaf; 5% in the advanced spherule stage.
+ maltose	90% with two fully developed leaves and root-hairs; 10% with one fully developed leaf, and one half-developed; root-hairs. Healthy.	10% with two fully developed leaves, forming the third, also roots; 80% with one fully developed and one half-developed leaf and root-hairs; 10% forming first leaf.

The practically negative results obtained on plain nutrient media without any sugar supply are in agreement with those of other investigators who dealt with the same subject. Although the seedlings in these solutions were still living when the data were taken, there is no doubt that they would gradually have died. This fact was proved also in a number of preliminary experiments performed in this laboratory. The conclusion that orchid seedlings grown under aseptic conditions on sugar-free media are not capable of germination is therefore highly plausible. It might be emphasized that a slight difference in the development in solution L from that in solution B was noticed, as is indicated in

the tables. While in solution B the embryos had reached only the small spherule stage and the leaf point was not even marked, those in solution L had passed through this stage and had a leaf point, although very weakly marked; some few of them had even tried to form a leaf, but apparently were unable to continue growing.

The best growth occurred on media supplied with maltose, especially in solution L. The seedlings grown on medium B were very well developed and healthy in appearance, but the protocorms were slender, the leaves narrow and lying at full length. The diameters of the protocorms in solution B were as much as 36 per cent shorter than of those grown in solution L. Referring to Knudson's experiments on symbiosis with different fungi on media supplied with starch, it is very probable that the favorable effect of certain fungi upon germination and development of orchid seeds might be ascribed to the fact that those fungi in question, by enzymatic processes, transform the starch to sugars, which are better digested by the orchid embryos, and that their metabolic processes render the medium more acid, thus favoring enzyme action. A similar explanation could be assumed in cases where a favorable effect of bacteria and yeasts had been reported. Knudson says: "When starch is provided as the organic matter in the culture media, there is no germination unless the fungus is supplied." The facts that no germination took place on starch-media not inoculated with a fungus, as reported by Knudson, and that the starch remained untouched, show distinctly that orchid seeds do not possess enzymes to transform the starch to sugar, and that they therefore are unable to use this food supply. Burgeff ('09) reports that the best growth of the symbiotic fungi was recorded on media containing agar, rain-water, and a small supply of starch. He also points out that for those fungi maltose was a better source of sugar than saccharose. From the fact that in starch hydrolysis maltose is the final product which can be transformed to glucose, also from the results obtained in the experiments reported in this paper, and finally, from the evidence that glucose is not an especially favorable sugar supply for orchid embryos, we may conclude that maltose is probably one of the best-suited foods for orchid seedlings. The probability is that

the hydrolysis of maltose to glucose furnishes energy to the growing plant. Additional credence is given this supposition by Bernard's ('09) report in which he states that the starch is transformed to sugar when the symbiotic fungus enters the cells of the germinating orchid seeds.

There is no doubt that the orchid seed contains a supply of sugar, although in very small amounts. In many tests made on seeds of Cattleya, Laelio-Cattleya, Brasso-Cattleya, etc., the author has always found reducing sugars. Burgeff (*l. c.*, p. 70–71) mentions that a yellow coloring matter goes into solution if orchid seeds are allowed to stand for several days in water; he supposes that this undetermined matter, which reduces Fehling's solution and therefore has the qualities of a sugar, attracts the symbiotic fungus. This undetermined reducing sugar might be considered the only source of carbohydrate for the germinating seed. If it is used up and no other suitable carbohydrate is available the embryo is doomed to die.

If we suppose the protocorms to be spheres and calculate the volume of those spheres from the average-measurements given in table 1, we will find a difference in volume of as much as 2.5 times. However, the smaller the volume the less is the assimilation. This difference in the ratio of assimilation indisputably has an influence upon the growth and development of the seedlings. It is a question whether the constitution of solution L causes this difference in development. Certainly the vigorous growth of the protocorms indicates a healthy development, since the nutrient material obtained from the nutrient solution is evidently not only sufficient to maintain growth, but there is undoubtedly a surplus of material which is stored in the tissue of the protocorm.

Also the pH concentration of the medium is of great importance, a fact which was brought into prominence by Knudson and proved by Clement. As has been mentioned above, in nutrient solutions with pH values of 5.2 and below the growth takes place in a normal fashion; above 5.2 growth is markedly retarded and signs of chlorosis appear. Hydrogen-ion concentrations of about 6.0 and above are very unfavorable for growth. Usually the seeds do not germinate at all, and if they do chlorosis takes place and the seeds die after they have reached the small spherule

stage. The favorable effect of a symbiotic fungus is explained by the additional fact that the fungus produces acid, thus changing the pH concentration of the less favorable medium to one more favorable, a fact also pointed out by Knudson, as mentioned above. This indicates that when the seed is planted under aseptic conditions on a maltose medium, for example, enzymes are present in small quantities and these in turn are stimulated or retarded in their activity by acid or basic conditions.

Since hydrochloric acid was used in order to adjust the pH concentration, the amount of chloride is higher in solution L than in solution B, which contains no chlorides in the original composition. Preliminary experiments have demonstrated that the effect of chlorine on growth is not of importance, and the effect on germination is problematical; likewise it is doubtful whether the treatment of seeds before planting (with chlorinated lime) has a stimulating effect upon germination. Seeds taken under aseptic conditions from a seed pod and planted without sterilization directly on the medium germinated as well as those treated before planting with the sterilizing solution in question. This, however, does not exclude the possibility of stimulating seed germination by *hypotonic* chloride solutions when Popoff's ('25) method is employed.

A comparative examination of the two nutrient solutions shows that there is, theoretically, 5 times more potasium in solution L; further 4 times as much phosphate, and about 16 per cent more nitrogen. At first it would seem to be impossible to apply a nutrient solution of such composition, but we must take into account the fact that a relatively large amount of salt is precipitated when the solution is subjected to sterilization and to titration with either acids or alkalies. That precipitate consists, as far as could be determined qualitatively in default of an equipment for quantitative analyses, of C, K, P, and Fe, and we may suppose that the difference is not as striking as it appeared from the first. We assume that the amount of nitrogen was increased slightly, the amount of both potassium and phosphorus, many times. What is the effect upon germination and growth? According to the records summarized in the tables the development in solution L was, in general, 5 to 10 per cent better than in solution B. The

seedlings appeared darker in color and had progressed relatively further in development. This effect might be ascribed to the larger dose of phosphorus and potassium. Maiwald ('23) and Schertz ('29) found, in experiments on potato and cotton plants, a correlation between phosphorus, potassium, and nitrogen and the formation of chloroplast pigments. Nitrogen increased the amount of pigments, large amounts of phosphorus produced more chloroplasts than potassium and less than nitrogen, large amounts of potassium evidently suppressed pigment formation. Stoklasa ('16) has proved that the potassium ion plays an important part in the processes of metabolism. Clements' ('28) studies on the nutrition of pea seeds indicate that KH_2PO_4 has a very marked influence on nitrogen assimilation, and also that a satisfactory balance between nitrogen and carbohydrates is of importance. The results of the experiments in solution L can be interpreted as the effect of large doses of potassium on the synthesis of proteins (Weever, '11) and the assimilation of nitrogen, an effect connected with an increase in assimilation of carbohydrates. It must be borne in mind, however, that assimilation is also dependent on the nature of the sugars and the presence of proper enzymes in the organism. The activity of enzymes, in turn, appears to be dependent to some extent on the pH concentration of the medium.

SUMMARY

1. Of the sugars used in these experiments maltose was found to be most favorable for the germination and growth of *Cattleya* seeds. According to their effect upon growth, the other sugars rank as follows: levulose, glucose, and saccharose.

2. The hydrogen-ion concentration is of great importance in germination and development of *Cattleya* seeds, much better growth occurring in solutions with a pH value between 4.8 and 5.2.

3. On media with a pH value at or above 5.6 germination and growth are retarded and in most instances chlorosis takes place. With a pH value of 6.0 and higher there is extreme chlorosis and the seedlings are unable to germinate.

4. On glucose and sucrose, the least favorable carbohydrate sources, the seedlings develop more slowly and chlorophyll is formed less rapidly, even at the most favorable pH concentration.

5. A nutrient solution, solidified with agar, of a composition different from Knudson's solution B was tried. The growth and development on this medium are better than on solution B.

6. Factors involved in this problem are discussed.

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Explanation of Plate

PLATE 42

Fig. 1. Cattleya seedlings No. 27 grown on medium L + 2 per cent maltose. × ⅚.

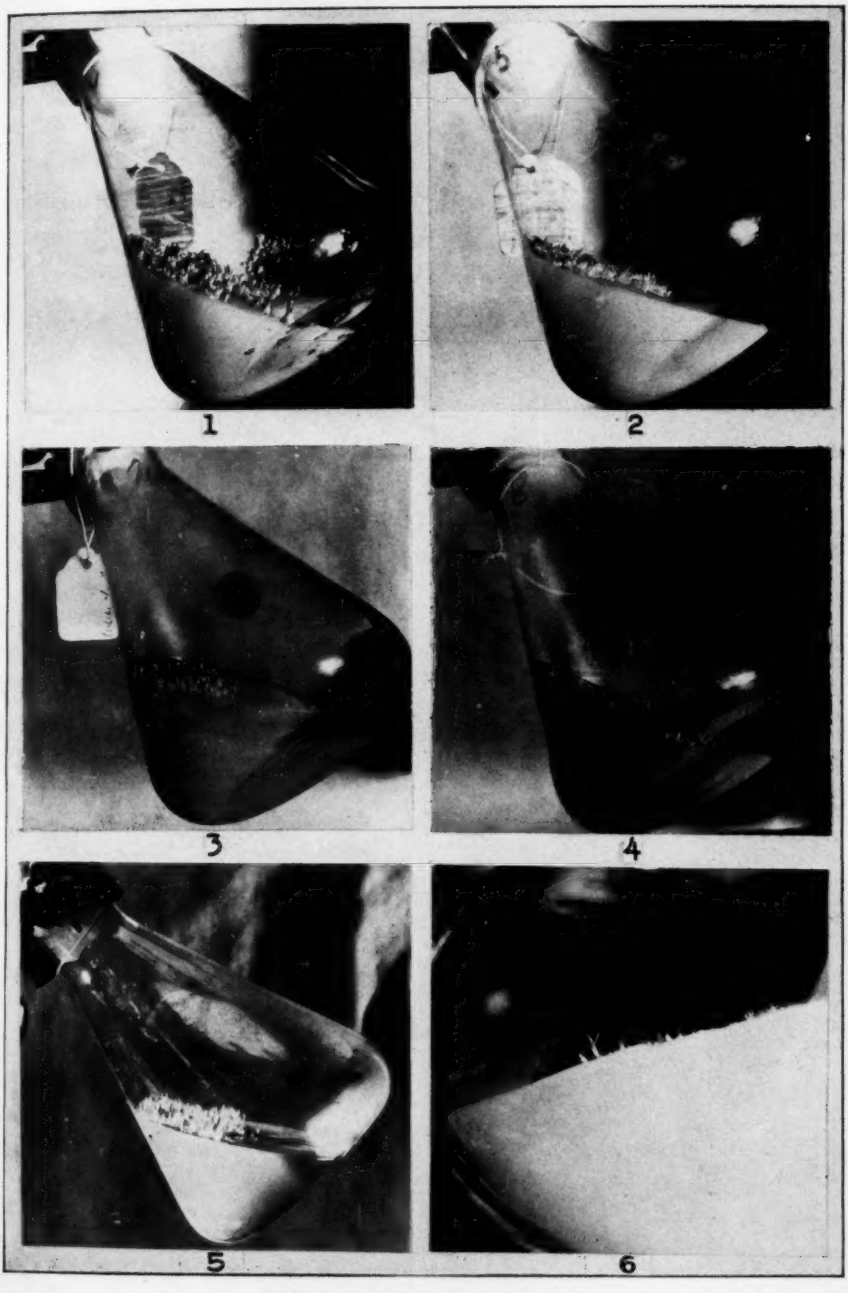
Fig. 2. Cattleya seedlings No. 27 grown on medium B + 2 per cent maltose. × ⅚.

Fig. 3. Cattleya seedlings No. 21 grown on medium L + 2 per cent sucrose. × ⅚.

Fig. 4. Cattleya seedlings No. 21 grown on medium B + 2 per cent sucrose. × ⅚.

Fig. 5. Cattleya seedlings No. 27 grown on medium L + 2 per cent maltose, showing chlorosis as consequence of a pH concentration of about 5.8. × ½.

Fig. 6. Cattleya seedlings No. 27 grown on medium L + 2 per cent sucrose; the first two seedlings on the left side are shown growing on the top of other seedlings. Natural size.



LA GARDE—ORCHID GERMINATION

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